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(54) Title: TETRODOTOXIN-SENSITIVE SODIUM CHANNEL α-SUBUNIT

(57) Abstract

DNA encoding for a voltage-gated, TTX-sensitive sodium channel is isolated. Also disclosed are polypeptide products of recombinant expression of these DNA sequences, expression vectors comprising the DNA sequence, and host cells transformed with these expression vectors. Other aspects of this invention are peptides whose sequences are based on the amino acid sequences deduced from these DNA sequences, antibodies specific for such proteins and peptides, procedures for detection and quantitation of such proteins and nucleic acids related thereto. Another aspect of this invention is the use of this voltage-gated, tetrodotoxin-sensitive sodium channel as a therapeutic target for compounds.

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Tetrodotoxin-Sensitive Sodium Channel α-Subunit

The present invention relates generally to sodium channel proteins and more particularly to a novel cloned α-subunit of a voltage-gated, tetrodotoxin-sensitive sodium channel protein. The present invention further relates to its production by recombinant technology and nucleic acid sequences encoding for this protein.

BACKGROUND OF THE INVENTION

The basic unit of information transmitted from one part of the nervous system to another is a single action potential or nerve impulse. The „transmission line" for these impulses is the axon, or nerve fiber. The electrical excitability of the nerve membrane has been shown to depend on the membrane's voltage-sensitive ionic permeability system that allows it to use energy stored in ionic concentration gradients. Electrical activity of the nerve is triggered by a depolarization of the membrane, which opens channels through the membrane that are highly selective for sodium ions, which are then driven inward by the electrochemical gradient. Of the many ionic channels, the voltage-gated or voltage-sensitive sodium channel is one of the most studied. It is a transmembrane protein that is essential for the generation of action potentials in excitable cells. An excellent review of sodium channels is presented in Catterall, TINS 16(12), 500-506 (1993).

The cDNAs for several Na^+ channels have been cloned and sequenced. Numa *et al.*, Annals of the New York Academy of Sciences 479, 338-355 (1986), describe cDNA from the electric organ of eel and two different ones from rat brain. Rogart, U.S. Patent No. 5,380,836, describes cDNA from rat cardiac tissue. See also Cribbs *et al.* Proc. Natl. Acad. Sci., 86, 8170-8174 (1989). A peripheral nerve sodium channel, referred to as PN1, has been detected based on sodium current studies and hybridization to a highly conserved sodium channel probe by D'Arcangelo *et al.*, J. Cell Biol. 122, 915-921 (1993). However, neither the DNA nor the protein were isolated and its complete nucleic acid and amino acid sequence were unidentified. A partial amino acid sequence was presented at the 23rd Annual Meeting of the Society for Neuroscience, November 7-12, 1993, Washington D.C., see Abstracts: Volume 19, Part 1: Abstract 121.7: "Nerve Growth Factor Treatment of PC12 Cells Induces the Expression of a Novel Sodium Channel Gene, Peripheral Nerve Type 1 (PN1)", by B.L. Moss, J. Toledo-Aral and G. Mandel.

A sodium channel gene abundantly expressed in neurons and glia, referred to as NaCh6, was detected in 1995 based on RNase protection assays, *in situ* hybridization and RT-PCR hybridization. See Schaller *et al.*, J. Neuroscience 15(5), 3231-3242 (1995).

These studies have shown that the amino acid sequence of the Na^+ channel has been conserved over a long evolutionary period. These studies have also revealed that the channel is a single polypeptide containing four internal repeats, or homologous domains (domains I-IV), having similar amino acid sequences. Each domain folds into six predicted transmembrane α-helices or segments: five are hydrophobic segments and one is highly charged with many lysine and arginine residues. This highly charged segment is the fourth transmembrane segment in each domain (the S4 segment) and is likely to be involved in voltage-gating. The positively charged side chains on the S4 segment are likely to be paired with the negatively charged side chains on the other five segments such that membrane depolarization could shift the position of one helix relative to the other, thereby opening the channel. Accessory subunits may modify the function of the channel.

Therapeutic utility in recombinant materials derived from the DNA of the numerous sodium channels have been discovered. For example, U.S. Patent No. 5,132,296 discloses purified Na^+ channels that have proven useful as therapeutic and diagnostic tools.

Isoforms of sodium channels are divided into „subfamilies". The term „isoform" is used to mean distinct but closely related sodium channel proteins, i.e., those having an amino acid homology of approximately 60-80%. These also show strong homology in functions. The term „subfamilies" is used to mean distinct sodium channels that have an amino acid homology of approximately 80-95%. Combinations of several factors are used to determine the distinctions within a subfamily, for example, the speed of a channel, chromosomal location, expression data, homology to other channels within a species and homology to a channel of the same subfamily across species. Another consideration is an affinity to tetrodotoxin („TTX"). TTX is a highly potent toxin from the puffer or fugu fish which blocks the conduction of nerve impulses along axons and in excitable membranes of nerve fibers. TTX binds to the Na^+ channel and blocks the flow of sodium ions.

Studies using TTX as a probe have shed much light on the mechanism and structure of Na^+ channels. There are three Na^+ channel subtypes that are defined by the affinity for TTX, which can be measured by the IC_{50} values: TTX-sensitive Na^+ channels ($IC_{50} \approx$

1 nM), TTX-insensitive N^+ channels ($IC_{50} \approx$ 1-5 μM), and TTX-resistant Na^+ channels ($IC_{50} \geq 100$ μM).

TTX-insensitive action potentials were first studied in rat skeletal muscle (Redfern *et al.*, Acta Physiol. Scand. 82, 70-78 (1971)). Subsequently, these action potentials were described in other mammalian tissues, including newborn mammalian skeletal muscle, mammalian cardiac muscle, mouse dorsal root ganglion cells in vitro and in culture, cultured mammalian skeletal muscle and L6 cells (Rogart, Ann. Rev. Physiol. 43, 711-725 (1980)).

Dorsal root ganglia neurons possess both TTX-sensitive ($IC_{50} \simeq 0.3$ nM) and TTX-resistant ($IC_{50} \simeq 100$ μM) sodium channel currents, as described in Roy *et al.*, J. Neurosci. 12, 2104-2111 (1992).

TTX-resistant sodium currents have also been measured in rat nodose and petrosal ganglia (Ikeda *et al.*, J. Neurophysiol. 55, 527-539 (1986) and Stea *et al.*, Neurosci. 47, 727-736 (1992)).

DESCRIPTION OF THE INVENTION

The present invention relates to novel sodium channel proteins. Specific embodiments include the α-subunit of such sodium channels that are TTX-sensitive.

In particular, the present invention relates to a purified and isolated DNA sequence encoding for a novel rat TTX-sensitive sodium channel protein and a splice variant thereof. The term "purified and isolated DNA" refers to DNA that is essentially free, i.e. contains less than about 30%, preferably less than about 10%, and even more preferably less than about 1% of the DNA with which the DNA of interest is naturally associated. Techniques for assessing purity are well known to the art and include, for example, restriction mapping, agarose gel electrophoresis, and CsCl gradient centrifugation. The term "DNA" is meant to include cDNA made by reverse transcription of mRNA or by chemical synthesis.

Specifically, the invention encompasses DNA having the engineered versions (discussed in detail below) of the nucleotide sequences set forth in SEQ ID NOS:1 and 2 designated herein as nerve sodium channel types 4 and the splice variant 4a (PN4 and PN4a). These versions of the PN4 and PN4a sequence were produced by removing most of the untranslated sequences of the PN4 and PN4a cDNA and cloned into expression

vectors for functional analysis. The longer „native" version of PN4 is shown in Fig. 3 (SEQ ID NO:7). The complete „native" base pair sequence of PN4a has the same sequence shown in Fig. 3 and labeled rPN4 with the 30 base pair insert after position 2050. The PN4 and PN4a DNA sequences comprise cDNA sequences that encode the α-subunit of novel voltage-gated, TTX-sensitive sodium channels, specifically the amino acid sequences set forth in Figs. 1 and 2 (SEQ ID NOS: 3 and 4). DNA sequences encoding the same or allelic variant or analog sodium channel protein polypeptides of the nervous system, through use of, at least in part, degenerate codons are also contemplated by this invention. The nucleotide sequences of SEQ ID NOS:1 and 2 correspond to the cDNAs from rat. A homology search provided that the closest related sodium channel is found in the fugu (puffer fish), with 92% homology. The next closest channels are rat brain types I and II, at 87.9% and rat brain type III at 87.3%. Homology to all other known channels drops off significantly thereafter.

Additionally, it is believed that the novel voltage-gated, TTX-sensitive sodium channel is also expressed in tissue of other mammalian species such as humans, and that the corresponding gene is highly homologous to the rat sequence. Therefore, the invention includes cDNA encoding a novel mammalian voltage-gated, TTX-sensitive sodium channel.

The invention not only includes the entire protein expressed by the cDNA sequences of SEQ ID NOS:1 and 2, but also includes protein fragments. These fragments can be obtained by cleaving the full length proteins or by using smaller DNA sequences or polynucleotides to express the desired fragment. Accordingly, the invention also includes polynucleotides that can be used to make polypeptides of about 10 to 1500, preferably 10 to 100, amino acids in length. The isolation and purification of such recombinant polypeptides can be accomplished by techniques that are well known in the art, for example preparative chromatographic separations or affinity chromatography. In addition, polypeptides can also be made by synthetic means which are well known in the art.

In general, sodium channels comprise an α- and two J-subunits. The J-subunits may modulate the function of the channel. However, since the α-subunit is all that is required for the channel to be fully functional, expression of the cDNA in SEQ ID NOS:1 and 2, will each provide a fully functional protein. The gene encoding the J_1-subunit in nerve tissue was found to be identical to that found in rat heart, brain and skeletal muscle. The cDNA of the J_1-subunit is not described herein as it is well known in the art (Isom *et al.*, Neuron 12, 1183-1194 (1994)). However, it is to be understood that by combining the

known sequence for the J_1-subunit with the α-subunit sequence described herein, one may obtain complete PN4 and PN4a rat voltage-gated, TTX-sensitive sodium channels.

Northern blot analysis indicates that PN4 and PN4a are each encoded by a ~7.5 kb/9.5 kb transcript. The nucleotide sequence analysis of the PN4 cDNA identifies a 5934-base open reading frame, shown in SEQ ID NO:1, starting at base 22. The nucleotide sequence analysis of the PN4a cDNA identifies a 5964-base open reading frame, shown in SEQ ID NO:2, also starting at base 22. The deduced amino acid sequence of PN4, shown in Fig. 1 (SEQ ID NO:3), exhibits the primary structural features of an α-subunit of a voltage-gated, TTX-sensitive sodium channel. Shown in Fig. 1 are the homologous domains (I-IV); the putative transmembrane segments (Sl-S6); the amino acid conferring sensitivity to TTX (Δ); potential cAMP-phosphorylation site (•); and potential N-glycosylation site (♦). The deduced amino acid sequence of PN4a, shown in Fig. 2 (SEQ ID NO:4), also exhibits the primary structural features of an α-subunit of a voltage-gated, TTX-sensitive sodium channel. Shown in Fig. 2 are the homologous domains (I-IV); the putative transmembrane segments (Sl-S6); the amino acid conferring sensitivity to TTX (Δ); potential cAMP-phosphorylation site (•); and potential N-glycosylation site (♦).

Reverse transcription-polymerase chain reaction (degenerate oligonucleotide-primed „RT-PCR“) analysis of RNA from the rat central and peripheral nervous systems, in particular from rat dorsal root ganglia („DRG“) was performed. Eight main tissue types were screened by RT-PCR for expression of the unique PN4 genes corresponding to positions 4646-5203 of SEQ ID NO:1. PN4 was present in five of the tissues studied: brain, spinal cord, DRG, nodose ganglia and superior cervical ganglia. PN4 was not present in the remaining tissues studied: sciatic nerve tissue, heart or skeletal muscle tissue.

Three main tissue types were screened by RT-PCR for expression of the unique PN4a genes corresponding to positions 1947-2135 of SEQ ID NO:2. PN4a was present in two of the tissues studied: spinal cord and DRG. PN4a was not present in brain tissue.

The invention also pertains to the cloning and functional expression in *Xenopus* oocytes of the novel PN4 and PN4a rat TTX-sensitive sodium channels. Specifically, the α-subunit of the sodium channels was cloned and expressed. Functional expression shows that PN4 and PN4a are voltage-gated, TTX-sensitive sodium channels with properties that are similar to other TTX-sensitive sodium channels.

Preferred aspects of this invention are PN4 cDNA sequences which encode for the novel mammalian TTX-sensitive sodium channel proteins that are expressed in brain, spinal cord, dorsal root ganglia, nodose ganglia and superior cervical ganglia but not in sciatic nerve, heart or skeletal muscle when assayed by the methods described herein, such as RT-PCR.

Also preferred aspects of this invention are PN4a cDNA sequences which encode for the novel mammalian TTX-sensitive sodium channel proteins that are expressed most strongly in DRG, with little expression in spinal cord and almost undetectable expression in brain when assayed by the methods described herein, such as RT-PCR.

cDNA sequences which encode for the novel PN4 TTX-sensitive sodium channel proteins that are predominantly expressed in the brain and spinal cord are also contemplated by this invention. cDNA sequences which encode for the novel PN4a TTX-sensitive sodium channel proteins that are predominantly expressed in the DRG are also contemplated by this invention.

The term „cDNA", or complementary DNA, refers to single-stranded or double-stranded DNA sequences obtained by reverse transcription of mRNA isolated from a donor cell. For example, treatment of mRNA with a reverse transcriptase such as AMV reverse transcriptase or M-MuLV reverse transcriptase in the presence of an oligonucleotide primer will furnish an RNA-DNA duplex which can be treated with RNase H, DNA polymerase, and DNA ligase to generate double-stranded cDNA. If desired, the double-stranded cDNA can be denatured by conventional techniques such as heating to generate single-stranded cDNA. The term „cDNA" includes cDNA that is a complementary copy of the naturally occurring mRNA as well as complementary copies of variants of the naturally occurring mRNA, that have the same biological activity. Variants would include, for example, insertions, deletions, sequences with degenerate codons and alleles. For example, PN4a is a splice variant of PN4, having a 10 amino acid insertion.

The term „cRNA" refers to RNA that is a copy of the mRNA transcribed by a cell. cRNA corresponding to mRNA transcribed from a DNA sequence encoding the α-subunit of a novel TTX-sensitive sodium channel protein is contemplated by this invention.

The present invention also includes expression vectors comprising the DNA or the cDNA described above, host cells transformed with these expression vectors capable of producing the sodium channel of the invention, and cDNA libraries comprising such host cells.

The term "expression vector" refers to any genetic element, e.g., a plasmid, a chromosome, a virus, behaving either as an autonomous unit of polynucleotide expression within a cell or being rendered capable of replication by insertion into a host cell chromosome, having attached to it another polynucleotide segment, so as to bring about the replication and/or expression of the attached segment. Suitable vectors include, but are not limited to, plasmids, bacteriophages and cosmids. Vectors will contain polynucleotide sequences which are necessary to effect ligation or insertion of the vector into a desired host cell and to effect the expression of the attached segment. Such sequences differ depending on the host organism, and will include promoter sequences to effect transcription, enhancer sequences to increase transcription, ribosomal binding site sequences and transcription and translation termination sequences.

The term "host cell" generally refers to prokaryotic or eukaryotic organisms and includes any transformable or transfectable organism which is capable of expressing a protein and can be, or has been, used as a recipient for expression vectors or other transferred DNA. Host cells can also be made to express protein by direct injection with exogenous cRNA translatable into the protein of interest. A preferred host cell is the *Xenopus* oocyte.

The term "transformed" refers to any known method for the insertion of foreign DNA or RNA sequences into a host prokaryotic cell. The term „transfected" refers to any known method for the insertion of foreign DNA or RNA sequences into a host eukaryotic cell. Such transformed or transfected cells include stably transformed or transfected cells in which the inserted DNA is rendered capable of replication in the host cell. They also include transiently expressing cells which express the inserted DNA or RNA for limited periods of time. The transformation or transfection procedure depends on the host cell being transformed. It can include packaging the polynucleotide in a virus as well as direct uptake of the polynucleotide, such as, for example, lipofection or microinjection. Transformation and transfection can result in incorporation of the inserted DNA into the genome of the host cell or the maintenance of the inserted DNA within the host cell in plasmid form. Methods of transformation are well known in the art and include, but are

not limited to, viral infection, electroporation, lipofection and calcium phosphate mediated direct uptake.

It is to be understood that this invention is intended to include other forms of expression vectors, host cells and transformation techniques which serve equivalent functions and which become known to the art hereto.

The term „cDNA library" refers to a collection of clones, usually in a bacteriophage, or less commonly in bacterial plasmids, containing cDNA copies of mRNA sequences derived from a donor cell or tissue.

In addition, the present invention contemplates recombinant polynucleotides, of about 15 to 20kb, preferably 10 to 15kb nucleotides in length, comprising a nucleic acid sequence derived from the DNA of the invention.

The term "polynucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modified, for example, by methylation and/or by capping, and unmodified forms of the polynucleotide.

The term "derived from" a designated sequence, refers to a nucleic acid sequence that is comprised of a sequence of approximately at least 6-8 nucleotides, more preferably at least 10-12 nucleotides, and, even more preferably, at least 15-20 nucleotides that correspond to, i.e., are homologous or complementary to, a region of the designated sequence. The derived sequence is not necessarily physically derived from the nucleotide sequence shown, but may be derived in any manner, including for example, chemical synthesis or DNA replication or reverse transcription, which are based on the information provided by the sequences of bases in the region(s) from which the polynucleotide is derived.

Further, the term "polynucleotide" is intended to include a recombinant polynucleotide, which is of genomic, cDNA, semisynthetic or synthetic origin which, by virtue of its origin or manipulation is not associated with all or a portion of the polynucleotide with which it is associated in nature and/or is linked to a polynucleotide other than that to which it is linked in nature.

The „native" version of PN4, and its splice variant PN4a, partially correspond to the sodium channel NaCh6 (described by Schaller *et al.* in J. Neurosci. 15, 3231-3242 (1995)) as shown in Figs. 3 and 4. In Figs. 3 and 4, base pair and amino acid sequences of the native PN4 and PN4a sodium channel cDNA clones include untranslated sequences. The published NaCh6 sequence does appear to not correctly provide the sodium channel sequence, and the sequences for PN4 and PN4a appear to represent the authentic sodium channel sequence for the following reasons:

First, most sodium channel gene coding regions, including PN4, begin with an eleven base pair sequence consisting of an out of frame ATG, followed by five base pairs downstream, followed by the ATG initiation codon for the coding region. The DNA sequence alignment (Fig. 3) shows a two base pair deletion in NaCh6 overlapping the second ATG, so that the normally out of frame, upstream ATG becomes the NaCh6 initiation codon, leading to a two amino acid insertion. Start and stop codons are underlined and primers are denoted by dashed lines with arrows.

Examination of the DNA sequence alignment (Fig. 3) shows that the bulk of the differences (residues in bold print) between the two sequences that would strongly influence protein function consist of a series of nine single base deletions in the Interdomain I/II region. These differences lead to a very different amino acid sequence, as shown in the amino acid alignment of Fig. 4, where the differences between the two sequences are again shown in bold print. The applicants' sequencing of multiple isolates resulting from the cloning of up to 1.5kb of the Interdomain I/II region by PCR repeatedly resulted in sequences which completely agreed with PN4 or PN4a sequences.

Comparison of PN4 and PN4a sequences to other sodium channel sequences shows a high degree of homology. For example, Fig. 5 is a comparison of PN4 and PN4a with NaCh6 and rat Brain type II in this region. Whereas PN4 and BrainII share about 50% identity in the region highlighted in bold, NaCh6 is almost completely different. The differences between BrainII and PN4 are underlined.

Also PCR was employed to look specifically for NaCh6. A sense primer common to both sequences (CAATCGTGGGCGCCCTAATC, corresponding to base pair 722-742 of NaCh6 and shown by dashed lines with an arrow in Fig. 3 at bases 884-904 of PN4) was paired with gene specific antisense primers (TGCTTTCATGCACTGGAATCCCTCT,

corresponding to base pair 1194-1170 of PN4, and TGCTTTACTGCACTGGAATCCTTCG, corresponding to base pair 1029-1005 of NaCh6; sequence differences between the two primers are underlined). The antisense primers prime at a three base pair deletion of NaCh6 relative to PN4 and overlap three other sequence differences, as shown in Fig. 3. A PCR product of the expected size (about 300 base pairs) was obtained with the PN4 specific antisense primer using pBK-CMV/75-1.4 DNA (described in the description of SEQ ID NO:2) and with rat Brain and rat DRG cDNA templates. No PCR products were obtained from these templates with the NaCh6 specific primer.

Any of the sequence differences between PN4 and NaCh6 could result in an inability of the NaCh6 gene to form a functional channel. However, some differences could be ascribed to „base calling." To verify the accuracy of the sequence the full length versions of PN4 and PN4a were sequenced. Of the amino acid differences between PN4 and NaCh6, it appears that the profound differences in the Interdomain I/II region are responsible for the lack of success in expression of the NaCh6 gene. The nine single base deletions in this region appear to shift the reading frame (see Figs. 3 and 4), leading to a „nonsense" peptide which lacks a number of highly conserved residues (Fig. 5) and which could sufficiently disrupt the structure of the protein to destroy its function.

The splice variant PN4a is similar to and occurs in a homologous position to that seen with rat type Brain1 and 1a channels (Schaller *et al.*, *J. Neurosci* 12, 1370-1381 (1992)). In each case it appears that the variants make use of the same 3′ splice acceptor sites but alternative 5′ sites. Rat BrainIII also has splice variants in this region, using the same 3′ splice site but using alternative 5′ sites more 5′ than the other channels. An amino acid comparison with other rat(r) and human(h) channels is shown below. Not all sodium channels have this splicing pattern.

rPN4	GRLLPE	....	AT.TEVE
rPN4a	GRLLPE	VKIDKAAT.DS	AT.TEVE
rBRAIN1	GQLLPE	VIIDKPATDDN	GTTTETE
rBRAIN1a	GQLLPE	....	GTTTETE
rPN1	GQLLPE	VIIDKATSDDS	GTTNQMR
hNE-Na	GQLLPE	....	GTTNQIH
rBRAIN2	GQLLPE	....	GTTTETE
rBRAIN3	....	....	GTTTETE
rCARDIAC	SYLLRP	MVLDRPP . . DT	TTPSEEP

It is interesting to note that rat PN1 is similar to PN4a whereas its human homologue, the neuroendocrine channel, hNE-Na, is similar to PN4. Perhaps each of these will be found to be one of a set of splice variants. Whereas the splicing patterns of BrainI, II, and III were found not to vary across a range of tissues (Schaller *et al.*, *J. Neurosci* 12, 1370-1381 (1992)), PN4 and PN4a show dramatic abundance differences. PN4 has a gradient of expression with high expression in brain, intermediate in spinal cord and relatively the least in DRG. PN4a is very low or undetectable in brain, a minor fraction of total PN4 expression in spinal cord, and nearly as abundant as PN4 in DRG.

Many uses of the invention exist, a few of which are described below.

1. Probe for human channel.

As mentioned above, it is believed that homologs of the novel rat TTX-sensitive sodium channel described herein are also expressed in mammalian nerve tissue, in particular, human tissue. The entire cDNAs of PN4 and PN4a rat sodium channels of the present invention can be used as a probe to discover whether novel PN4 and PN4a voltage-gated, TTX-sensitive sodium channels exist in human nerve tissue and, if they do, to aid in isolating the cDNAs for the human protein.

The human homologues of the rat TTX-sensitive PN4 and PN4a channels can be cloned using a human DRG cDNA library. Human DRG are obtained at autopsy. The frozen tissue is homogenized and the RNA extracted with guanidine isothiocyanate (Chirgwin *et al.*, Biochemistry 18, 5294-5299 (1979)). The RNA is size-fractionated on a sucrose gradient to enrich for large mRNAs because the sodium channel α-subunits are encoded by large (7-11 kb) transcripts. Double-stranded cDNA is prepared using the SuperScript Choice cDNA kit (GIBCO BRL) with either oligo(dT) or random hexamer primers. EcoRI adapters are ligated onto the double-stranded cDNA which is then phosphorylated. The cDNA library is constructed by ligating the double-stranded cDNA into the bacteriophage-lambda ZAP II vector (Stratagene) followed by packaging into phage particles.

Phage are plated out on 150 mm plates on a lawn of XLI-Blue MRF′ bacteria (Stratagene) and plaque replicas are made on Hybond N nylon membranes (Amersham). Filters are hybridized to rat PN4 and PN4a cDNA probes by standard procedures and detected by autoradiography or chemiluminescence. The signal produced by the rat PN4

and PN4a probes hybridizing to positive human clones at high stringency should be stronger than obtained with rat brain sodium channel probes hybridizing to these clones. Positive plaques are further purified by limiting dilution and re-screened by hybridization or PCR. Restriction mapping and polymerase chain reaction will identify overlapping clones that can be assembled by standard techniques into the full-length human homologue of rat PN4 and PN4a. The human clone can be expressed by injecting cRNA transcribed *in vitro* from the full-length cDNA clone into *Xenopus* oocytes, or by transfecting a mammalian cell line with a vector containing the cDNA linked to a suitable promoter.

2. Probe for Obtaining Molecular Data.

The polynucleotides of the invention can be bound to a reporter molecule to form a polynucleotide probe useful for Northern and Southern blot analysis and *in situ* hybridization.

The term "reporter molecule" refers to a chemical entity capable of being detected by a suitable detection means, including, but not limited to, spectrophotometric, chemiluminescent, immunochemical, or radiochemical means. The polynucleotides of this invention can be conjugated to a reporter molecule by techniques well known in the art. Typically the reporter molecule contains a functional group suitable for attachment to or incorporation into the polynucleotide. The functional groups suitable for attaching the reporter group are usually activated esters or alkylating agents. Details of techniques for attaching reporter groups are well known in the art. See, for example, Matthews et al., Anal. Biochem., 151, 205-209 (1985) and Engelhardt *et al.*, European Patent Application No. 0 302 175.

3. Antibodies Against PN4 and PN4a.

The polypeptides of the invention are highly useful for the development of antibodies against PN4 and PN4a. Such antibodies can be used in affinity chromatography to purify recombinant sodium channel proteins or polypeptides, or they can be used as a research tool. For example, antibodies bound to a reporter molecule can be used in histochemical staining techniques to identify other tissues and cell types where PN4 and PN4a are present, or they can be used to identify epitopic or functional regions of the sodium channel protein of the invention.

The antibodies can be monoclonal or polyclonal and can be prepared by techniques that are well known in the art. Polyclonal antibodies are prepared as follows: an immunogenic conjugate comprising PN4, PN4a or a fragment thereof, optionally linked to a carrier protein, is used to immunize a selected mammal such as a mouse, rabbit, goat, etc. Serum from the immunized mammal is collected and treated according to known procedures to separate the immunoglobulin fraction.

Monoclonal antibodies are prepared by standard hybridoma cell technology based on that reported by Köhler and Milstein in Nature 256, 495-497 (1975): spleen cells are obtained from a host animal immunized with the PN4 or PN4a protein or a fragment thereof, optionally linked to a carrier. Hybrid cells are formed by fusing these spleen cells with an appropriate myeloma cell line and cultured. The antibodies produced by the hybrid cells are screened for their ability to bind to expressed PN4 or PN4a proteins.

A number of screening techniques well known in the art, such as, for example, forward or reverse enzyme-linked immunosorbent assay screening methods may be employed. The hybrid cells producing such antibodies are then subjected to recloning and high dilution conditions in order to select a hybrid cell that secretes a homogeneous population of antibodies specific to either the PN4 or PN4a protein.

In addition, antibodies can be raised by cloning and expressing nucleotide sequences or mutagenized versions thereof coding at least for the amino acid sequences required for specific binding of natural antibodies, and these expressed proteins used as the immunogen. Antibodies may include the complete immunoglobulin or a fragment thereof. Antibodies may be linked to a reporter group such as is described above with reference to polynucleotides.

4. Therapeutic Targets for Disorders.

The present invention also includes the use of the novel voltage-gated, TTX-sensitive sodium channel α-subunit as a therapeutic target for compounds to treat disorders of the nervous system including, but not limited to, epilepsy, stroke injury, brain injury, allodynia, hyperalgesia, diabetic neuropathy, traumatic injury and AIDS-associated neuropathy. The invention allows for the manipulation of genetic materials by recombinant technology to produce polypeptides that possess the structural and functional characteristics of the novel voltage-gated, TTX-sensitive sodium channel α-subunit found in nerve tissue, particularly in sensory nerves. Site directed mutagenesis can be used to

provide such recombinant polypeptides. For example, synthetic oligonucleotides can be specifically inserted or substituted into the portion of the gene of interest to produce genes encoding for and expressing a specific mutant. Random degenerate oligonucleotides can also be inserted and phage display techniques can be used to identify and isolate polypeptides possessing a functional property of interest.

5. Designing Therapeutics based on Inhibiting PN4 and PN4a and assays thereof.

The present invention is also directed to inhibiting the activity of PN4 in brain, spinal cord, DRG, nodose ganglia and superior cervical ganglia tissues. This invention is also directed to inhibiting the activity of PN4a in spinal cord and DRG tissues. However, it is to be understood that further studies may reveal that PN4 and PN4a are present in other tissues, and as such, those tissues can also be targeted areas. For example, the detection of PN4 mRNA in nodose ganglia suggests that PN4 may conduct TTX-sensitive sodium currents in this and other sensory ganglia of the nervous system.

In addition, it has been found that proteins not normally expressed in certain tissues, are expressed in a disease state. Therefore, this invention is intended to encompass the inhibition of PN4 and PN4a in tissues and cell types where the protein is normally expressed, and in those tissues and cell types where the protein is only expressed during a disease state.

The invention also pertains to an assay for inhibitors of the novel TTX-sensitive sodium channel protein comprising contacting a compound suspected of being an inhibitor with expressed sodium channel and measuring the activity of the sodium channel. The compound can be a substantially pure compound of synthetic origin combined in an aqueous medium, or the compound can be a naturally occurring material such that the assay medium is an extract of biological origin, such as, for example, a plant, animal, or microbial cell extract. PN4 and PN4a activity can be measured by methods such as electrophysiology (two electrode voltage clamp or single electrode whole cell patch clamp), guanidinium ion flux assays and toxin-binding assays. An "inhibitor" is defined as generally that amount that results in greater than 50% decrease in PN4 or PN4a activity, preferably greater than 70% decrease in PN4 or PN4a activity, more preferably, greater than 90% decrease in PN4 or PN4a activity.

6. Designing and Screening for Additional Therapeutics.

Another significant characteristic of PN4 is that it is TTX-sensitive. It is believed that TTX-sensitive sodium channels play a key role in transmitting nerve impulses relating to sensory inputs such as pain and pressure. This will also facilitate the design of therapeutics that can be targeted to a specific area such as nerve tissue.

Additionally, the recombinant protein of the present invention can be used to screen for potential therapeutics that have the ability to inhibit the sodium channel of interest. In particular, it would be useful to inhibit selectively the function of sodium channels in nerve tissues responsible for transmitting pain and pressure signals without simultaneously affecting the function of sodium channels in other tissues such as heart and muscle. Such selectivity would allow for the treatment of pain without causing side effects due to cardiac or neuromuscular complications. Therefore, it would be useful to have DNA sequences coding for sodium channels that are selectively expressed in nerve tissue.

7. Pain Reliever.

Sodium channels in nerve tissue play a large role in the transmission of nerve impulses, and therefore are instrumental in understanding neuropathic pain transmission. Neuropathic pain falls into two categories: allodynia, where a normally non-painful stimulus becomes painful, and hyperalgesia, where a usually normal painful stimulus becomes extremely painful. The ability to inhibit the activity of these sodium channels, i.e., reduce the conduction of nerve impulses, will affect the nerve's ability to transmit pain. Selective inhibition of sodium channels in sensory neurons such as dorsal root ganglia will allow the blockage of pain impulses without complicating side effects caused by inhibition of sodium channels in other tissues such as brain and heart. In addition, certain diseases are caused by sodium channels that produce impulses at an extremely high frequency. The ability to reduce the activity of the channel can then eliminate or alleviate the disease. Accordingly, potential therapeutic compounds can be screened by methods well known in the art, to discover whether they can inhibit the activity of the recombinant sodium channel of the invention. See Barram *et al.*, Naun-Schmiedeberg's archives of Pharmacology, 347, 125-132 (1993) and McNeal *et al.*, J. Med. Chem., 28, 381-388 (1985). For similar studies with the acetyl choline receptor, see, Claudio *et al.*, Science 238, 1688-1694 (1987).

Accordingly, the present invention encompasses a method of alleviating pain by inhibiting the activity of the novel TTX-sensitive sodium channel comprising administering a therapeutically effective amount of a compound having an IC_{50} in the range of 0.1-50 nM, preferably within the range of 1-25 nM, and most preferably within the range of 1-5 nM.

Potential therapeutic compounds are identified based on their ability to inhibit the activity of PN4 and PN4a. Therefore, the aforementioned assay can be used to identify compounds having a therapeutically effective IC_{50}.

The term „IC_{50}“ refers to the concentration of a compound that is required to inhibit by 50% the activity of expressed PN4 or PN4a when activity is measured by electrophysiology, flux assays and toxin-binding assays, as mentioned above.

The basic molecular biology techniques employed in accomplishing features of this invention, such as RNA, DNA and plasmid isolation, restriction enzyme digestion, preparation and probing of a cDNA library, sequencing clones, constructing expression vectors, transforming cells, maintaining and growing cell cultures and other general techniques are well known in the art, and descriptions of such techniques can be found in general laboratory manuals such as Molecular Cloning: A Laboratory Manual by Sambrook, *et al.* (Cold Spring Harbor Laboratory Press, 2nd edition, 1989). Accordingly, the following examples are merely illustrative of the techniques by which the invention can be practiced.

BRIEF DESCRIPTION OF THE SEQ ID'S AND FIGURES

SEQ ID NO:1 depicts an engineered version of the nucleotide cDNA sequence encoding the rat TTX-sensitive peripheral nerve sodium channel type 4 („PN4“). This version lacks most of the untranslated sequences, thereby comprising a 5934-base open reading frame, from nucleotide residue 22 of the XhoI-HindIII clone, the start site of translation, and ending at residue 5956.

SEQ ID NO:2 depicts an engineered version of the nucleotide cDNA sequence encoding the rat TTX-sensitive peripheral nerve sodium channel type 4a („PN4a“). This version lacks most of the untranslated sequences, thereby comprising a 5964-base open reading frame, beginning at nucleotide residue 22 of the XhoI-HindIII clone, the start site of translation, and ending at residue 5986. The 30 base pair insert is found at positions 2014-2043.

Fig. 1 (SEQ ID NO:3) depicts the deduced amino acid sequence of PN4, represented in the single-letter amino acid code. Shown in Fig. 1 are the homologous domains (I-IV); the

putative transmembrane segments (SI-S6); the amino acid conferring sensitivity to TTX (Δ); potential cAMP-phosphorylation site (•); and potential N-glycosylation site (♦).

Fig. 2 (SEQ ID NO:4) depicts the deduced amino acid sequence of PN4a, represented in the single-letter amino acid code. Shown in Fig. 2 are the homologous domains (I-IV); the putative transmembrane segments (SI-S6); the amino acid conferring sensitivity to TTX (Δ); potential cAMP-phosphorylation site (•); and potential N-glycosylation site (♦).

Fig. 3 aligns the base pair sequences of the NaCh6 and the „native" version of the PN4 sodium channel cDNA clones (SEQ ID NO:7), including untranslated sequences, depicting the differences in bold. Start and stop codons are underlined and primers are denoted by dashed lines with arrows.

Fig. 4 aligns the amino acid sequences of the PN4a, PN4 and NaCh6 sodium channel cDNA clones of Fig. 3, depicting the differences in bold.

Fig. 5 is a comparison of the conserved region Interdomain I/II between PN4a, PN4, NaCh6 and BrainII sodium channels. Differences between PN4 (and PN4a) and NaCh6 are shown in bold type and differences between BrainII and PN4 are underlined.

SEQ ID NO:5 depicts the 696 nucleotide cDNA sequence encoding the novel probe CNaD4-2 used to identify the novel sodium channels of the invention.

SEQ ID NO:6 depicts the deduced amino acid sequence of probe CNaD4-2, represented in the single-letter amino acid code.

Fig. 6 depicts the cloning map of PN4 and PN4a.

Fig. 7 shows the properties of currents produced in *Xenopus* oocytes by injection of PN4 cRNA. Fig. 7a shows the current produced by sodium channels expressed in oocyte, Fig. 7b shows the current-voltage relationship.

Fig. 8a and 8b show steady state inactivation of sodium currents produced by PN4 in *Xenopus* oocytes.

Fig. 9 demonstrates the effects of the β_1 and β_2 subunits upon PN4 function in *Xenopus* oocytes. Fig. 9a shows currents produced when the PN4 I subunit is injected alone; Fig. 9b is with the β_1 subunit; Fig. 9c is with the β_2 subunit; and Fig. 9d is with both the β_1 and β_2 subunits.

Abbreviations

BSA	bovine serum albumin
Denhardt's solution	0.02% BSA, 0.02% polyvinyl-pyrrolidone, 0.02% Ficoll (0.1 g BSA, 0.1 g Ficoll and 0.1 g polyvinylpyrrolidone per 500 ml)
DRG	dorsal root ganglia
EDTA	Ethylenediaminetetraacetic acid, tetrasodium salt
MEN	20 mM MOPS, 1 mM EDTA, 5 mM sodium acetate, pH 7.0
MOPS	3-(N-morpholino)propanesulfonic acid (Sigma Chemical Company)
PN3	peripheral nerve sodium channel type 3
PNS	peripheral nervous system
SDS	sodium dodecyl sulfate
SSC	150 mM NaCl, 15 mM sodium citrate, pH 7.0
SSPE	80 mM NaCl, 10 mM sodium phosphate, 1 mM ethylenediaminetetraacetate, pH 8.0
TEV	two electrode voltage clamp
TTX	tetrodotoxin (Sigma Chemical Company)
UTR	untranslated region

EXAMPLES

Each step employed in obtaining the DNA of the novel sodium channel of the invention is described in the detailed examples below. The following is an overview of the steps. Example 1 describes how a novel probe, CNaD4-2, was obtained by designing primers based on known sodium channels. Example 2 describes the construction and screening of a cDNA library with CNaD4-2 to obtain the 3′ end of the novel sodium channel of the invention. Then, a known primer was employed to obtain the 5′ end of the DNA of the invention. Example 3 describes how RT-PCR was employed to span the gap,

between the 3′ and 5′ ends obtained from the cDNA library. This resulted in a 798 base pair sequence and a splice variant thereof, having a 828 base pair sequence. Example 4 describes assembling the clones into two full-length cDNA clones in expression vectors. The cloning map is illustrated in Fig. 6. Example 5 discusses the tissue distribution and localization accomplished by RT-PCR. Example 6 discusses the northern analysis of mRNA. Example 7 discloses obtaining expression data from *Xenopus* oocytes, and localization by RT-PCR.

Materials

The plasmid pBK-CMV was obtained from Stratagene (La Jolla, CA); plasmid Litmus 29 was obtained from New England Biolabs (Beverly, MA); the oocyte expression vector plasmid pBSTAcIIr was constructed from pBSTA (described by Goldin *et al.*, in Methods in Enzymology (Rudy & Iverson, eds.) 207, 279-297) by insertion of a synthetic oligonucleotide linker; the mammalian cell expression vector plasmid pCI-neo was obtained from Promega (Madison, WI); plasmid pCRII was obtained from Invitrogen, San Diego, CA. Competent *E. Coli* cell lines STBL2™ and SURE® were obtained from GIBCO/BRL and Stratagene, respectively.

EXAMPLE 1: Identification of a novel channel fragment

A novel probe used to identify the novel sodium channels was obtained as follows. Degenerate oligonucleotide primers were designed based on the homologies between known sodium channels in domain IV and used to perform RT-PCR on RNA isolated from rat DRG. The domain IV PCR products were cloned into pCRII, transformed into *E. coli* and single colonies isolated. DNA sequence of the inserts of several of these colonies was obtained, including the following novel sequence from clone pCRII/CNaD4-2 of SEQ ID NO:5, identified as CNaD4-2. SEQ ID NO:6 depicts the deduced amino acid sequence of probe CNaD4-2, represented in the single-letter amino acid code.

CNaD4-2 can be made with standard PCR techniques.

EXAMPLE 2: Construction and screening of cDNA library from rat DRG with probe CNaD4-2

EcoRI-adapted cDNA was prepared from normal adult male Sprague-Dawley rat DRG poly(A)+ RNA using the SuperScript Choice System (GIBCO BRL). cDNA (>4kb) was selected by sucrose gradient fractionation as described by Kieffer (Gene 109, 115-119 (1991)). The cDNA was then ligated into the Zap Express vector (Stratagene), and

packaged with the Gigapack II XL lambda packaging extract (Stratagene). Plate lysates were prepared and screened by PCR using CNaD4-2 specific primers (ACACTCAGAGCAAGCAGATGG and TCCCTGGGTGCTCTTTGTCCA, corresponding to bases 32 to 52 and 569 to 589 of SEQ ID NO:5, respectively). Phage from one positive lysate were screened by filter hybridization with a ^{32}P-labeled probe (the 700 base pair EcoRI insert from CNaD4-2). Filters were hybridized in 50% formamide, 5X SSPE, 5X Denhardt's solution, 0.5% SDS, 250μg/ml sheared, denatured salmon sperm DNA, and 50mM sodium phosphate at 42°C and washed in 0.5X SSC, 0.1% SDS at 50°C. Positive clones were excised in vivo into pBK-CMV using the ExAssist/XLOLR system (Stratagene).

Approximately 95% of these clones contained sodium channel sequence under standard screening stringency conditions. The number of clones that are retrieved that contain sodium channel sequence can be increased with increased stringency conditions and careful analysis and interpretation of data. It is well known in the art when screening for a particular type of DNA sequence, other types of DNA sequences will also be hybridized, depending on the specificity of the probe. Here, with the careful designed probe of the invention, the approximate 95% „hit" rate, makes this fragment an exceptionally good sodium channel probe.

One of these clones, pBK-CMV/PN4.10-1, contained sequence of the CNaD4-2 channel from domain II through the 3′ UTR. The position of the pBK-CMV/PN4.10-1 fragment in the PN4 and PN4a cloning map is shown in Fig. 6. In Fig. 6, ATG is the start codon, TAC is the stop codon and ∇ is the position of the PN4a splice insert.

A degenerate primer designed for sodium channels in domain I (ACCAACTG[T/C]GT[G/A]TT[T/C]ATGAC) was paired with a PN4 specific primer from the domain II region of pBK-CMV/PN4.10-1 (CAGCAGCTACAGTGGCTACA). These primers amplified a ca 1.5kb fragment from rat brain and from rat DRG which was shown by sequencing to represent much of the 5′ end of PN4, thus verifying that the primers would work for screening the library. The primers were then used to screen plate lysates of the DRG cDNA library by PCR. Positive lysates were plated and individual plaques picked and screened by PCR using the same primers. Positive clones were excised in vivo into pBK-CMV using the ExAssist/XLOLR system. One of these, pBK-CMV/75-1.4, was found to contain PN4 sequence from the 5′ UTR to the interdomain I/II region, but

not to domain II, possibly due to rearrangement during the excision process. The position of the pBK-CMV/75-1.4 fragment in the PN4 and PN4a cloning map is shown in Fig. 6.

EXAMPLE 3: Cloning the interdomain I/II region

The gap between pBK-CMV/75-1.4 and pBK-CMV/PN4.10-1 was cloned by RT-PCR on rat DRG and brain total RNA using specific primers: AAAGAGGCCGAGTTCAAGGC (a base pair sequence of pBK-CMV/75-1.4) and TGTCCTTCCGTCCGTAGG (a base pair sequence of pBK-CMV/PN4.10-1). PCR products were cloned into plasmid pCRII and sequenced. Two distinct sequences, FA-2 and FA-7 (see Fig. 6), were cloned from DRG. These were found to be identical except for the presence of a 30 base pair insert (found at base pairs 2014-2043 in SEQ ID NO:2, and depicted by an upside triangle at the position of insertion in FA-7, FJ-13 and PN4a in Fig. 6), with sequence identity to pBK-CMV/75-1.4 and pBK-CMV/PN4.10-1 in the regions where they overlap. RT-PCR on rat brain RNA yielded only clones which lacked the 30 base pair insert. This insert is homologous to a splice variant of the NaChI channel (NaChIa) and likely results from alternative 5′ splice site usage (Schaller *et al., J. Neurosci* 12, 1370-1381 (1992)).

Additional RT-PCR was performed on rat DRG RNA using primers TTCATGGGAACCTTCGAAAC (a base pair sequence of pBK-CMV/75-1.4) and GAACGATGCAGATGGTGATGGCTAA (a base pair sequence of pBK-CMV/PN4.10-1). The 1.5kb PCR product was cloned into pCRII; six out of twenty isolates were positive for the 30 base pair insert variant by PCR. The sequence obtained for one of these, FJ-13, position shown in Fig. 6, was identical to that expected from the sequences of pBK-CMV/75-1.4, FA-7, and pBK-CMV/PN4.10-1, thus confirming that these clones all originated from the same transcript.

EXAMPLE 4: Assembly of full-length PN4 clones in expression vectors

Unsuccessful attempts have been made to create and stabilize full-length sodium channel cDNA sequences. In US Patent No. 5,380,836, the cDNA sequence for a rat cardiac sodium channel protein was contained in three separate plasmids. In order to create full-length functional PN4 genes, the 5′ end was modified: suitable restriction sites were added and the upstream out-of-frame initiation codon was removed. The modified pBK-CMV/75-1.4 and FA-2 sequences were fused together, then combined with the remaining portion of PN4 from pBK-CMV/PN4.10-1 in suitable expression vectors. PCR was employed to assemble the 5′ portion of PN4 from the initiation codon to domain II. A

1.43kb PCR fragment was generated from pBK-CMV/75-1.4 using the following primers: (1) GAAGCTCGAGCCCGGCAAGAGAAG**ATG**GCAGCGCGG (Xho-I Srf-I restriction sites underlined, initiation codon in bold, PN4 homology in italics, a base pair sequence of pBK-CMV/75-1.4) and primer (2) CTCGGAGAGCCTACCCCATC (a base pair sequence of pBK-CMV/75-1.4, and a base pair sequence of FA-2). A 0.69kb PCR fragment was generated from FA-2 using primer (3) AGAAGGGGAAGATGGGGTAGG (a base pair sequence of FA-2, and a base pair sequence of pBK-CMV/75-1.4) and primer (4) ATTCTGTCCTTCCGTCCGTAG (a base pair sequence of FA-2, and a base pair sequence of pBK-CMV/PN4.10-1). These fragments were gel purified and then a small fraction of each was combined as template in a further PCR reaction using primers (1) and (4). The fragments share a 31 base pair region of overlap at their 3′ and 5′ ends respectively, and therefore can act as primers to fuse the two fragments together (Horton *et al., Gene* 77, 61-68 (1991)). The 2.1kb PCR product was cloned into pCRII and several isolates were sequenced, one of which, FD-8, had the expected sequence. The position of FD-8 in the PN4 and PN4a clones is shown in the cloning map of Fig. 6.

To facilitate cloning into pBSTA and pCI-neo, it was determined to introduce an XbaI site at the 3′ end. To accomplish this, the PN4 domain II to 3′ UTR region was subcloned from pBK-CMV/PN4.10-1 from the EcoRI site of the vector to the HindIII site 14 base pairs from the PN4 stop codon into EcoRI plus HindIII digested Litmus 29. The resulting clone was labeled FC-1. The position of FC-1 in the PN4 and PN4a clones is shown in the cloning map of Fig. 6.

To assemble the full length PN4, the 5′ portion was subcloned from FD-8 as a 2.0kb Xho I-Eco NI fragment together with the 3′ portion from FC-1 as a 4.0kb Eco NI-Xba-I fragment into Xho-I plus Xba-I digested pBSTAcIIr. One of the resulting isolates was found to have the correct sequence and was named pBSTAcIIr_PN4(FU-7A).

The splice variant, PN4a, was assembled by replacing the 1.3kb Sph I - Acc I region of pBSTAcIIr_PN4(FU-7A) with the corresponding fragment from FJ-13, to form pBSTAcIIr_PN4a(FZA-3), and confirmed by DNA sequencing.

PN4 and PN4a were recloned into pCI-neo as 6.0kb Xho-I to Xba-I fragments to form pCI-neo-PN4(GAII-1) and pCI-neo-PN4a(GCII-2), respectively, and confirmed by DNA sequencing. The sequences of the coding regions as cloned in the oocyte and

mammalian cell expression vectors of PN4 and PN4a are SEQ ID NO:1 and SEQ ID NO:2, respectively.

Growth of fragments of PN4 or PN4a was accomplished under standard conditions, however growth of plasmids containing full length constructs of PN4 and PN4a (in pCIneo or pBSTAcIIr) could not be accomplished without use of special growth media, conditions, and *E. coli* strains. The following proved to be optimal: (1) use of *E. coli* STBL2™ for primary transformation following ligation reactions; for large scale culturing the primary transformants in STBL2™ cells were used, but secondary transformants in SURE® cells were used later if necessary. These *E. coli* strains have altered genotypes which allow the stable propagation of plasmids containing unstable inserts. (2) Solid media was 1/2x FM (see below) plus either 1x YENB (Bacto Yeast Extract, 0.75%, Bacto Nutrient Broth, 0.8%; Sharma and Schimke, Biotechniques 20, 42-44 (1996)), 1x YET (Bacto Yeast Extract, 0.75%, Bacto Tryptone, 0.8%), or 1x LB (Tryptone, 1%, Yeast Extract, 0.5%, NaCl, 0.5%), plus 15g/L agar. (3) Liquid media optimally was 1x FM plus 1/2x LB. (4) Carbenicillin, 100μg/ml, was used for all media, as it is metabolized less rapidly than ampicillin. However, carbenicillin may be used within the range of 50-200 μg/ml; and more preferably within the range of 75-125 μg/ml. (5) Temperature for growth should be no greater than 30°C, usually 28°C; this necessitated longer growth periods than normally employed, from 36 to 48 hours.

The recipe for 2x Freezing Medium (2xFM) is K2HPO4, 12.6g; Na3Citrate, 0.9g; MgSO4.7H2O, 0.18g; (NH4)2SO4, 1.8g; KH2PO4, 3.6g; Glycerol, 88g; H2O, qs to 1L.

2xFM and the remaining media components are prepared separately, sterilized by autoclaving, cooled to at least 60°C, and added together to form the final medium. Carbenicillin is prepared at 25mg/ml H2O and sterilized by filtration. 2xFM was first described for preparation of frozen stocks of bacterial cells (Practical Methods in Molecular Biology, Schleif, R.F. and Wensink, P.C., Springer-Verlag, New York (1981) pp201-202).

EXAMPLE 5: Tissue distribution by RT-PCR

Brain, spinal cord, DRG, nodose ganglia, superior cervical ganglia, sciatic nerve, heart and skeletal muscle tissue were isolated from anesthetized, normal adult male Sprague-Dawley rats and were stored at -80°C. RNA was isolated from each tissue using RNAzol (Tel-Test, Inc.). Random-primed cDNA was reverse transcribed from 500ng of

RNA from each tissue. The CNaD4-2 specific primers ACACTCAGAGCAAGCAGATGG and TCCCTGGGTGCTCTTTGTCCA (see above) defined a 558 base pair amplicon and would not discriminate between PN4 and PN4a. Thermal cycler parameters were 30 s/94°C, 30 s/64°C, 1 min/72°C (24 cycles (confirmation experiment: 34 cycles)); 30 s/94°C, 30 s/64°C, 5 min/72°C (1 cycle). A positive control (pCRII/CNaD4-2) and a no-template control were also included. cDNA from each tissue was also PCR amplified using primers specific for glyceraldehyde-3-phosphate dehydrogenase to demonstrate template viability, as described by Tso *et al.*, Nucleic Acid Res. 13, 2485-2502 (1985).

Tissue distribution profile of PN4 by analysis of RNA from selected rat tissues by RT-PCR was as follows:

Tissue	RT-PCR (35 cycles)
Brain	+++++
Spinal cord	+++
DRG	++
Nodose ganglia	++
Superior cervical ganglia	+
Sciatic nerve	-
Heart	-
Skeletal muscle	-

PN4 was also detected after only 25 cycles (24 + 1) in the same five tissues as above in the same relative abundance.

Since PN4 differs from PN4a by only 30 base pairs, a new sense primer, GGTGGACTGCAACGGCGTA (corresponding to the same base pair sequences of FA-2 and FA-7), was employed. RT-PCR using this primer together with primer ATTCTGTCCTTCCGTCCGTAG (primer 4 above) gave amplicons of 159 base pairs from PN4 and 189 base pairs from PN4a. Thermal cycler parameters were 1 min/95°C; 20sec/94°C, 30 sec/60°C, 1 min/72°C, 8 cycles; 20sec/94°C, 30 sec/58°C, 1 min/72°C, 27 cycles; 3 min/72°C. PN4a was nearly as abundant as PN4 in DRG, much less abundant than PN4 in spinal cord, and almost undetectable in brain. This correlates well with cloning data; based on sequenced, cloned RT-PCR fragments which included the 30 base pair insert

region, PN4a was found in 40% of isolates from DRG (9/24), but not found from brain (0/4).

Tissue	RT-PCR (35 cycles)	
	PN4	PN4a
Brain	+++++	(+ / -)
Spinal cord	+++	+
DRG	++	++

EXAMPLE 6: Northern Analysis of mRNA from rat DRG

Lumbar DRG #4 and #5 (L4 and L5), brain and spinal cord were removed from anesthetized adult male Sprague-Dawley rats under a dissecting microscope. The tissues were frozen in dry ice and homogenized with a Polytron homogenizer; the RNA was extracted by the guanidine isothiocyanate procedure (Chomczynksi *et al.*, Anal. Biochemistry 162, 156-159 (1987)). Total RNA (5 µg of each sample) was dissolved in MEN buffer containing 50% formamide, 6.6% formaldehyde and denatured at 65°C for 5-10 minutes. The RNA was electrophoresed through a 0.8% agarose gel containing 8.3% formaldehyde in MEN buffer. The electrode buffer was MEN buffer containing 3.7% formaldehyde; the gel was run at 50 V for 12-18 hour.

After electrophoresis, the gel was rinsed in 2xSSC and the RNA was transferred to a Duralose membrane (Stratagene) with 20xSSC by capillary action; the membrane was baked under vacuum at 80°C for 1 hour. The membrane was prehybridized in 50% formamide, 5xSSC, 50 mM sodium phosphate, pH 7.1, 1x Denhardt's solution, 0.5% SDS, and sheared, heat-denatured salmon sperm DNA (1 mg/ml) for 16 hour at 42°C. The membrane was hybridized in 50% formamide, 5xSSC, 50 mM sodium phosphate, pH 7.1, 1x Denhardt's solution, 0.5% SDS, and sheared, heat-denatured salmon sperm DNA (200 µg/ml) with a ^{32}P- labeled cRNA probe (ca. 1-3x10^6 cpm/ml). The probe was the cloned fragment, CNaD4-2, which contains the Domain 4 sequence of PN4 sodium channel α-subunit sequence. The probe was hybridized for 18 hour at 42°C. The cRNA probe was synthesized by excising and subcloning the fragment into pBluescript KS+ vector, purchased from Stratagene. The cRNA was transcribed *in vitro* using T3 RNA polymerase, purchased from Promega, after linearizing the plasmid with XbaI, purchased from Boehringer Mannheim. Protocols for each procedure mentioned above can be found in Molecular Cloning: A Laboratory Manual by Sambrook *et al.* (Cold Spring Harbor Laboratory Press, 2nd edition, 1989).

The membrane was washed three times with 2xSSC, 0.1% SDS at room temperature for 20 minutes and then washed once with 0.1xSSC, 0.1% SDS at 68°C for 30 minutes. The filter was exposed against Kodak X-omat AR film at -80°C with intensifying screens for up to two weeks.

Size markers, including ribosomal 18S and 28S RNAs and RNA markers (GIBCO BRL), were run in parallel lanes of the gel. Their positions were determined by staining the excised lane with ethidium bromide (0.5 μg/ml) followed by photography under UV light. The CNaD4-2 probe hybridized to RNA from the brain, cerebellum, dorsal and ventral horn of the spinal cord with sizes of 11 kb, 9.5 kb, 7.5 kb, and 6.5 kb, estimated on the basis of their positions relative to the standards.

Bands of the same size were detected in a blot containing total RNA from DRG from neuropathic pain model. However, no signal was detected with RNA from naive DRG.

PN4 constitutes a subfamily of novel sodium channel genes; these genes are different from those detectable with other probes (e.g., PEAF8 and PN3 probes), as discussed in copending application no. 08/511,828. Sequence comparison of PN4 with NaCh6 (mRNA size = 9.5kb) (Schaller *et al.*, J. Neurosci. 15, 3231-3242 (1995)) and cardiac-specific sodium channel for which only a partial sequence is available (mRNA size = 7kb) (Sills *et al.*, J. Clin. Invest. 84, 331-336 (1989)) indicates that these genes share a higher homology among themselves than with members of other sodium channel subfamilies such as the brain-type sodium channels, TTX-insensitive cardiac sodium channel and the TTX-resistant PN3 sodium channel.

Semiquantitation of the signal intensity of the various bands detected in the blot containing RNAs from the neuropathic pain model indicated that the level of 7.5kb transcript was upregulated ~35 fold as compared with the DRG from the sham operated side on day 1 after the surgery, wherein the sciatic nerve was ligated with four loose ligatures causing a constriction injury. None of the other transcripts detected by the CNaD4-2 probe was regulated so dramatically. By day 2, the regulation was reduced to ~5 fold as compared with the sham operated side. The experiment was performed with DRG pooled from 6 rats.

This experimental data suggests that PN4, or its splice variant, PN4a, is involved in the pathophysiology of neuropathic pain.

EXAMPLE 7: Expression of full length clone in *Xenopus* oocytes

cRNA was prepared from PN4 subcloned into pBSTA using a T7 in vitro transcription kit (Ambion, mMessage mMachine) and was injected into stage V and VI *Xenopus* oocytes using a Nanojector (Drummond), as described in Goldin, supra. After 1.5 days at 20°C, the oocytes were impaled with agarose-cushion electrodes (0.3-0.8 MOhm) and voltage-clamped with a Geneclamp 500 amplifier (Axon Instruments) in TEV mode. See Schreibmayer *et al.*, Pflugers Arch. 426, 453-458 (1994).

Stimulation and recording were controlled by a computer running pClamp (Axon Instruments) (Kegel *et al.* J. Neurosci. Meth. 12, 317-330 (1982)). Oocytes were perfused with a solution containing: 81 mM NaCl, 2 mM KC1, 1 mM $MgCl_2$, 0.3 mM $CaCl_2$, 20 mM Hepes-NaOH, pH 7.5. The data collected is shown in the Figs. 7-9 and described on the following pages.

Fig. 7a shows the currents produced from a PN4 sodium channel expressed in a *Xenopus* oocyte using the Geneclamp P/-4 leak subtraction, filtered at 5 kHz with a 4-pole Bessel filter, and sampled at 50 kHz. Test pulses for TTX c/r were to 0 mV for 5 ms at 0.033 Hz. The x-axis denotes time in milliseconds.

Fig. 7b illustrates the voltage to current relationship of a PN4 sodium channel expressed in a *Xenopus* oocyte.

Fig. 8a and b show steady state inactivation of sodium currents produced by PN4 in *Xenopus* oocytes. In Fig. 8a the x-axis denotes time in milliseconds. In Fig. 8b the x-axis is conditioning potential in millivolts and the y-axis is current in TA.

Fig. 9 demonstrates the effects of the $ß_1$ and $ß_2$ subunits upon PN4 function in *Xenopus* oocytes. Shown are currents produced when the PN4 I subunit is injected (a) alone; (b) with the $ß_1$ subunit; (c) with the $ß_2$ subunit; and (d) with both the $ß_1$ and $ß_2$ subunits. The x-axis in each of these figures denotes time in milliseconds. As these figures show, the inactivation kinetics of a functionally active PN4 channel are accelerated by the $ß_1$ subunit. No obvious effects are seen with the $ß_2$ subunit.

As is seen in Fig. 7a and b expression of PN4 and PN4a produced an inward current with slow inactivation kinetics, similar to that of the rBIIa (Patton *et al.*. Neuron 7, 637-647 (1991)) and rSkM1 α-subunits expressed in the absence of the β_1-subunit. In the expression of PN4, 0.2 ng of cRNA gave $1.4 \pm 0.19\ \mu A$ (n = 9). In the expression of PN4a, 0.1 ng gave $1.8 \pm 0.23\ \mu A$ (n = 6). Co-injection of rCNβ_1 (1 ng/oocyte) with PN4 cRNA accelerated inactivation kinetics of the channel. as seen in Fig. 9a and b. This agreed with data obtained with rBIIa and hSCNβ_1.

For steady state inactivation, 10 second prepulses were used. In the steady state inactivation of PN4, $V1/2 = -70.7 \pm 0.71$ mV, $k = 5.5 \pm 0.55$ mV (n = 3). In the steady state inactivation of PN4a, $V1/2 = -73.3 \pm 0.97$ mV, $k = 5.5 \pm 0.28$ mV (n = 4). Leak currents were measured during long pulses to -100 mV and -120 mV, and the test currents corrected assuming that the leak currents had a linear current-voltage relationship. An inactivation of -70 mV is similar to most sodium channels.

TEVC activation data for PN4a was $V1/2 = -23 \pm 2.7$ mV, $k = -6.4 \pm 0.75$ mV (n = 3).

TTX IC 50 was found to be 1.0 ± 0.60 nM (n = 2).

Sodium channels are distinctively sensitive or insensitive to neurotoxins such as TTX. The TTX-sensitive brain and skeletal muscle sodium channels are blocked by nanomolar TTX concentrations, whereas the TTX-insensitive cardiac sodium channels are blocked by micromolar TTX concentrations. In rat heart sodium channel 1 (rh1), Cys^{374} is a critical determinant of TTX-insensitivity, as shown in Satin *et al.*, Science 256, 1202-1205(1992); in the TTX-sensitive rBI, rBII, rBIII, and rSkM1, the corresponding residue is either Phe or Tyr. When expressed in *Xenopus* oocytes, the PN4 sodium current is sensitive to TTX ($IC_{50} \geq 1$ nM).

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: F. HOFFMANN-LA ROCHE AG

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(I) TELEX: 962292/965542 hlr ch

(ii) TITLE OF INVENTION: Novel Cloned Tetrodotoxin-Sensitive Sodium Channel I-Subunit And A Splice Variant Thereof

(iii) NUMBER OF SEQUENCES: 7

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: Apple Macintosh

(C) OPERATING SYSTEM: System 7.1 (Macintosh)

(D) SOFTWARE: Word 5.0

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5977 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(F) TISSUE TYPE: Dorsal root ganglia

(G) CELL TYPE: Peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6007 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(F) TISSUE TYPE: Dorsal root ganglia

(G) CELL TYPE: Peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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4501 AAGAAGTACT ACAATGCCAT GAAAAAGCTG GGCTCCAAGA AGCCACAGAA GCCCATCCCC
4561 CGACCCTTGA ACAAAATCCA AGGGATTGTC TTTGATTTCG TCACTCAACA AGCCTTTGAC
4621 ATTGTGATCA TGATGCTCAT CTGCCTTAAC ATGGTGACAA TGATGGTGGA GACAGACACT
4681 CAGAGCAAGC AGATGGAGAA CATTCTTTAC TGGATTAATC TGGTCTTTGT CATCTTCTTC
4741 ACCTGCGAGT GTGTGCTCAA AATGTTTGCC TTGAGACACT ACTATTTCAC CATTGGCTGG
4801 AACATCTTTG ACTTTGTGGT GGTCATCCTC TCCATTGTGG GAATGTTCCT GGCTGATATC
4861 ATTGAGAAGT ACTTCGTCTC CCCAACCCTA TTCCGAGTTA TCCGATTGGC CCGTATTGGG
4921 CGCATCTTGC GTCTGATCAA GGGCGCCAAA GGGATCCGCA CCCTGCTCTT TGCCTTAATG
4981 ATGTCGCTGC CCGCCCTGTT CAACATCGGC CTCCTGCTCT TCCTCGTCAT GTTCATCTTC
5041 TCCATTTTTG GCATGTCCAA CTTCGCATAC GTGAAGCACG AGGCCGGCAT TGACGACATG
5101 TTCAACTTCG AGACATTTGG CAACAGCATG ATCTGTTTGT TCCAGATCAC AACGTCTGCT
5161 GGCTGGGATG GCCTGCTGCT GCCAATCCTG AACCGCCCCC CTGACTGCAG CTTGGACAAA
5221 GAGCACCCAG GGAGTGGCTT CAAAGGGGAC TGTGGGAACC CCTCGGTGGG CATCTTCTTC
5281 TTTGTGAGCT ACATCATCAT CTCCTTCCTG ATTGTGGTGA ACATGTACAT CGCCATCATC
5341 CTGGAGAACT TCAGCGTGGC CACCGAGGAG AGCGCCGACC CTCTGAGTGA GGATGACTTC
5401 GAGACTTTCT ATGAGATCTG GGAGAAGTTT GACCCAGACG CCACCCAGTT CATCGAGTAC
5461 TGTAAGCTGG CAGACTTTGC CGACGCCCTG GAGCACCCGC TCCGAGTACC CAAGCCCAAC
5521 ACCATCGAGC TCATCGCCAT GGACCTGCCC ATGGTGAGCG GAGATCGCAT CCACTGCTTG
5581 GACATCCTTT TCGCCTTCAC CAAGCGAGTC CTGGGAGACA GTGGGGAGTT GGACATCCTG
5641 CGGCAGCAGA TGGAGGAGCG GTTCGTGGCA TCCAATCCTT CCAAAGTGTC TTACGAGCCT
5701 ATCACAACCA CTCTGCGGCG CAAGCAGGAG GAGGTGTCTG CAGTGGTCCT GCAGCGTGCC
5761 TACAGGGGAC ACTTGGCTAG GCGGGGCTTC ATCTGCAGAA AGATGGCCTC CAACAAGCTG
5821 GAGAATGGAG GCACACACAG AGACAAGAAG GAGAGCACCC CGTCCACAGC CTCCCTCCCC
5881 TCTTACGACA GCGTCACAAA GCCAGACAAG GAGAAGCAGC AGCGTGCGGA GGAGGGCAGA

5941 AGGGAAAGAG CCAAGAGGCA AAAAGAGGTC AGGGAGTCCA AGTGCTAGAG GAGGGGAAAG
6001 GAAGCTT

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1978 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:________

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES (although, functionally expressed)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(F) TISSUE TYPE: dorsal root ganglia

(G) CELL TYPE: peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met	Ala	Ala	Arg	Leu	Leu	Ala	Pro	Pro	Gly	Pro	Asp	Ser	Phe	Lys
				5					10					15
Pro	Phe	Thr	Pro	Glu	Ser	Leu	Ala	Asn	Ile	Glu	Arg	Arg	Ile	Ala
				20					25					30
Glu	Ser	Lys	Leu	Lys	Lys	Pro	Pro	Lys	Ala	Asp	Gly	Ser	His	Arg
				35					40					45
Glu	Asp	Asp	Glu	Asp	Ser	Lys	Pro	Lys	Pro	Asn	Ser	Asp	Leu	Glu
				50					55					60
Ala	Cly	Lys	Ser	Leu	Pro	Phe	Ile	Tyr	Gly	Asp	Ile	Pro	Gln	Gly
				65					70					75
Leu	Val	Ala	Val	Pro	Leu	Glu	Asp	Phe	Asp	Pro	Tyr	Tyr	Leu	Thr
				80					85					90
Gln	Lys	Thr	Phe	Val	Val	Leu	Asn	Arg	Gly	Lys	Thr	Leu	Phe	Arg
				95					100					105
Phe	Ser	Ala	Thr	Pro	Ala	Leu	Tyr	Ile	Leu	Ser	Pro	Phe	Asn	Leu
				110					115					120
Ile	Arg	Arg	Ile	Ala	Ile	Lys	Ile	Leu	Ile	His	Ser	Val	Phe	Ser
				125					130					135

Met Ile Ile Met Cys Thr Ile Leu Thr Asn Cys Val Phe Met Thr
140 145 150

Phe Ser Asn Pro Pro Glu Trp Ser Lys Asn Val Glu Tyr Thr Phe
155 160 165

Thr Gly Ile Tyr Thr Phe Glu Ser Leu Val Lys Ile Ile Ala Arg
170 175 180

Gly Phe Cys Ile Asp Gly Phe Thr Phe Leu Arg Asp Pro Trp Asn
185 190 195

Trp Leu Asp Phe Ser Val Ile Met Met Ala Tyr Val Thr Glu Phe
200 205 210

Val Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu
215 220 225

Arg Ala Leu Lys Thr Ile Ser Val Ile Pro Gly Leu Lys Thr Ile
230 235 240

Val Gly Ala Leu Ile Gln Ser Val Lys Lys Leu Ser Asp Val Met
245 250 255

Ile Leu Thr Val Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu
260 265 270

Gln Leu Phe Met Gly Asn Leu Arg Asn Lys Cys Val Val Trp Pro
275 280 285

Ile Asn Phe Asn Glu Ser Tyr Leu Glu Asn Gly Thr Arg Gly Phe
290 295 300

Asp Trp Glu Glu Tyr Ile Asn Asn Lys Thr Asn Phe Tyr Met Val
305 310 315

Pro Gly Met Leu Glu Pro Leu Leu Cys Gly Asn Ser Ser Asp Ala
320 325 330

Gly Gln Cys Pro Glu Gly Phe Gln Cys Met Lys Ala Gly Arg Asn
335 340 345

Pro Asn Tyr Gly Tyr Thr Ser Phe Asp Thr Phe Ser Trp Ala Phe
350 355 360

Leu Ala Leu Phe Arg Leu Met Thr Gln Asp Tyr Trp Glu Asn Leu
365 370 375

Tyr Gln Leu Thr Leu Arg Ala Ala Gly Lys Thr Tyr Met Ile Phe
380 385 390

Phe Val Leu Val Ile Phe Val Gly Ser Phe Tyr Leu Val Asn Leu
395 400 405

Ile	Leu	Ala	Val	Val	Ala	Met	Ala	Tyr	Glu	Glu	Gln	Asn	Gln	Ala
				410					415					420
Thr	Leu	Glu	Glu	Ala	Glu	Gln	Lys	Glu	Ala	Glu	Phe	Lys	Ala	Met
				425					430					435
Leu	Glu	Gln	Leu	Lys	Lys	Gln	Gln	Glu	Glu	Ala	Gln	Ala	Ala	Ala
				440					445					450
Met	Ala	Thr	Ser	Ala	Gly	Thr	Val	Ser	Glu	Asp	Ala	Ile	Glu	Glu
				455					460					465
Glu	Gly	Glu	Asp	Gly	Val	Gly	Ser	Pro	Arg	Ser	Ser	Ser	Glu	Leu
				470					475					480
Ser	Lys	Leu	Ser	Ser	Lys	Ser	Ala	Lys	Glu	Arg	Arg	Asn	Arg	Arg
				485					490					495
Lys	Lys	Arg	Lys	Gln	Lys	Glu	Leu	Ser	Glu	Gly	Glu	Glu	Lys	Gly
				500					505					510
Asp	Pro	Glu	Lys	Val	Phe	Lys	Ser	Glu	Ser	Glu	Asp	Gly	Met	Arg
				515					520					525
Arg	Lys	Ala	Phe	Arg	Leu	Pro	Asp	Asn	Arg	Ile	Gly	Arg	Lys	Phe
				530					535					540
Ser	Ile	Met	Asn	Gln	Ser	Leu	Leu	Ser	Ile	Pro	Gly	Ser	Pro	Phe
				545					550					555
Leu	Ser	Arg	His	Asn	Ser	Lys	Ser	Ser	Ile	Phe	Ser	Phe	Arg	Gly
				560					565					570
Pro	Gly	Arg	Phe	Arg	Asp	Pro	Gly	Ser	Glu	Asn	Glu	Phe	Ala	Asp
				575					580					585
Asp	Glu	His	Ser	Thr	Val	Glu	Glu	Ser	Glu	Gly	Arg	Arg	Asp	Ser
				590					595					600
Leu	Phe	Ile	Pro	Ile	Arg	Ala	Arg	Glu	Arg	Arg	Ser	Ser	Tyr	Ser
				605					610					615
Gly	Tyr	Ser	Gly	Tyr	Ser	Gln	Cys	Ser	Arg	Ser	Ser	Arg	Ile	Phe
				620					625					630
Pro	Ser	Leu	Arg	Arg	Ser	Val	Lys	Arg	Asn	Ser	Thr	Val	Asp	Cys
				635					640					645
Asn	Gly	Val	Val	Ser	Leu	Ile	Gly	Pro	Gly	Ser	His	Ile	Gly	Arg
				650					655					660
Leu	Leu	Pro	Glu	Ala	Thr	Thr	Glu	Val	Glu	Ile	Lys	Lys	Lys	Gly
				665					670					675

Pro	Gly	Ser	Leu	Leu	Val	Ser	Met	Asp	Gln	Leu	Ala	Ser	Tyr	Gly
				680					685					690
Arg	Lys	Asp	Arg	Ile	Asn	Ser	Ile	Met	Ser	Val	Val	Thr	Asn	Thr
				695					700					705
Leu	Val	Glu	Glu	Leu	Glu	Glu	Ser	Gln	Arg	Lys	Cys	Pro	Pro	Cys
				710					715					720
Trp	Tyr	Lys	Phe	Ala	Asn	Thr	Phe	Leu	Ile	Trp	Glu	Cys	His	Pro
				725					730					735
Tyr	Trp	Ile	Lys	Leu	Lys	Glu	Ile	Val	Asn	Leu	Ile	Val	Met	Asp
				740					745					750
Pro	Phe	Val	Asp	Leu	Ala	Ile	Thr	Ile	Cys	Ile	Val	Leu	Asn	Thr
				755					760					765
Leu	Phe	Met	Ala	Met	Glu	His	His	Pro	Met	Thr	Pro	Gln	Phe	Glu
				770					775					780
His	Val	Leu	Ala	Val	Gly	Asn	Leu	Val	Phe	Thr	Gly	Ile	Phe	Thr
				785					790					795
Ala	Glu	Met	Phe	Leu	Lys	Leu	Ile	Ala	Met	Asp	Pro	Tyr	Tyr	Tyr
				800					805					810
Phe	Gln	Glu	Gly	Trp	Asn	Ile	Phe	Asp	Gly	Phe	Ile	Val	Ser	Leu
				815					520					825
Ser	Leu	Met	Glu	Leu	Ser	Leu	Ala	Asp	Val	Glu	Gly	Leu	Ser	Val
				830					835					840
Leu	Arg	Ser	Phe	Arg	Leu	Leu	Arg	Val	Phe	Lys	Leu	Ala	Lys	Ser
				845					850					855
Trp	Pro	Thr	Leu	Asn	Met	Leu	Ile	Lys	Ile	Ile	Gly	Asn	Ser	Val
				860					865					870
Gly	Ala	Leu	Gly	Asn	Leu	Thr	Leu	Val	Leu	Ala	Ile	Ile	Val	Phe
				875					880					885
Ile	Phe	Ala	Val	Val	Gly	Met	Gln	Leu	Phe	Gly	Lys	Ser	Tyr	Lys
				890					895					900
Glu	Cys	Val	Cys	Lys	Ile	Asn	Gln	Glu	Cys	Lys	Leu	Pro	Arg	Trp
				905					910					915
His	Met	Asn	Asp	Phe	Phe	His	Ser	Phe	Leu	Ile	Val	Phe	Arg	Val
				920					925					930
Leu	Cys	Gly	Glu	Trp	Ile	Glu	Thr	Met	Trp	Asp	Cys	Met	Glu	Val
				935					940					945

Ala	Gly	Gln	Ala	Met	Cys	Leu	Ile	Val	Phe	Met	Met	Val	Met	Val
				950					955					960
Ile	Gly	Asn	Leu	Val	Val	Leu	Asn	Leu	Phe	Leu	Ala	Leu	Leu	Leu
				965					970					975
Ser	Ser	Phe	Ser	Ala	Asp	Asn	Leu	Ala	Ala	Thr	Asp	Asp	Asp	Gly
				980					985					990
Glu	Met	Asn	Asn	Leu	Gln	Ile	Ser	Val	Ile	Arg	Ile	Lys	Lys	Gly
				995					1000					1005
Val	Ala	Trp	Thr	Lys	Val	Lys	Val	His	Ala	Phe	Met	Gln	Ala	His
				1010					1015					1020
Phe	Lys	Gln	Arg	Glu	Ala	Asp	Glu	Val	Lys	Pro	Leu	Asp	Glu	Leu
				1025					1030					1035
Tyr	Glu	Lys	Lys	Ala	Asn	Cys	Ile	Ala	Asn	His	Thr	Gly	Val	Asp
				1040					1045					1050
Ile	His	Arg	Asn	Gly	Asp	Phe	Gln	Lys	Asn	Gly	Asn	Gly	Thr	Thr
				1055					1060					1065
Ser	Gly	Ile	Gly	Ser	Ser	Val	Glu	Lys	Tyr	Ile	Ile	Asp	Glu	Asp
				1070					1075					1080
His	Met	Ser	Phe	Ile	Asn	Asn	Pro	Asn	Leu	Thr	Val	Arg	Val	Pro
				1085					1090					1095
Ile	Ala	Val	Gly	Glu	Ser	Asp	Phe	Glu	Asn	Leu	Asn	Thr	Glu	Asp
				1100					1105					1110
Val	Ser	Ser	Glu	Ser	Asp	Pro	Glu	Gly	Ser	Lys	Asp	Lys	Leu	Asp
				1115					1120					1125
Asp	Thr	Ser	Ser	Ser	Glu	Gly	Ser	Thr	Ile	Asp	Ile	Lys	Pro	Glu
				1130					1135					1140
Val	Glu	Glu	Val	Pro	Val	Glu	Gln	Pro	Glu	Glu	Tyr	Leu	Asp	Pro
				1145					1150					1155
Asp	Ala	Cys	Phe	Thr	Glu	Gly	Cys	Val	Gln	Arg	Phe	Lys	Cys	Cys
				1160					1165					1170
Gln	Val	Asn	Ile	Glu	Glu	Gly	Leu	Gly	Lys	Ser	Trp	Trp	Ile	Leu
				1175					1180					1185
Arg	Lys	Thr	Cys	Phe	Leu	Ile	Val	Glu	His	Asn	Trp	Phe	Glu	Thr
				1190					1195					1200
Phe	Ile	Ile	Phe	Met	Ile	Leu	Leu	Ser	Ser	Gly	Ala	Leu	Ala	Phe
				1205					1210					1215

Glu	Asp	Ile	Tyr	Ile	Glu	Gln	Arg	Lys	Thr	Ile	Arg	Thr	Ile	Leu
				1220					1225					1230
Glu	Tyr	Ala	Asp	Lys	Val	Phe	Thr	Tyr	Ile	Phe	Ile	Leu	Glu	Met
				1235					1240					1245
Leu	Leu	Lys	Trp	Thr	Ala	Tyr	Gly	Phe	Val	Lys	Phe	Phe	Thr	Asn
				1250					1255					1260
Ala	Trp	Cys	Trp	Leu	Asp	Phe	Leu	Ile	Val	Ala	Val	Ser	Leu	Val
				1265					1270					1275
Ser	Leu	Ile	Ala	Asn	Ala	Leu	Gly	Tyr	Ser	Glu	Leu	Gly	Ala	Ile
				1280					1285					1290
Lys	Ser	Leu	Arg	Thr	Leu	Arg	Ala	Leu	Arg	Pro	Leu	Arg	Ala	Leu
				1295					1300					1305
Ser	Arg	Phe	Glu	Gly	Met	Arg	Val	Val	Val	Asn	Ala	Leu	Val	Gly
				1310					1315					1320
Ala	Ile	Pro	Ser	Ile	Met	Asn	Val	Leu	Leu	Val	Cys	Leu	Ile	Phe
				1325					1330					1335
Trp	Leu	Ile	Phe	Ser	Ile	Met	Gly	Val	Asn	Leu	Phe	Ala	Gly	Lys
				1340					1345					1350
Tyr	His	Tyr	Cys	Phe	Asn	Glu	Thr	Ser	Glu	Ile	Arg	Phe	Glu	Ile
				1355					1360					1365
Asp	Ile	Val	Asn	Asn	Lys	Thr	Asp	Cys	Glu	Lys	Leu	Met	Glu	Gly
				1370					1375					1380
Asn	Ser	Thr	Glu	Ile	Arg	Trp	Lys	Asn	Val	Lys	Ile	Asn	Phe	Asp
				1385					1390					1395
Asn	Val	Gly	Ala	Gly	Tyr	Leu	Ala	Leu	Leu	Gln	Val	Ala	Thr	Phe
				1400					1405					1410
Lys	Gly	Trp	Met	Asp	Ile	Met	Tyr	Ala	Ala	Val	Asp	Ser	Arg	Lys
				1415					1420					1425
Pro	Asp	Glu	Gln	Pro	Asp	Tyr	Glu	Gly	Asn	Ile	Tyr	Met	Tyr	Ile
				1430					1435					1440
Tyr	Phe	Val	Ile	Phe	Ile	Ile	Phe	Gly	Ser	Phe	Phe	Thr	Leu	Asn
				1445					1450					1455
Leu	Phe	Ile	Gly	Val	Ile	Ile	Asp	Asn	Phe	Asn	Gln	Gln	Lys	Lys
				1460					1465					1470
Lys	Phe	Gly	Gly	Gln	Asp	Ile	Phe	Met	Thr	Glu	Glu	Gln	Lys	Lys
				1475					1480					1485

Tyr	Tyr	Asn	Ala	Met	Lys	Lys	Leu	Gly	Ser	Lys	Lys	Pro	Gln	Lys
				1490					1495					1500
Pro	Ile	Pro	Arg	Pro	Leu	Asn	Lys	Ile	Gln	Gly	Ile	Val	Phe	Asp
				1505					1510					1515
Phe	Val	Thr	Gln	Gln	Ala	Phe	Asp	Ile	Val	Ile	Met	Met	Leu	Ile
				1520					1525					1530
Cys	Leu	Asn	Met	Val	Thr	Met	Met	Val	Glu	Thr	Asp	Thr	Gln	Ser
				1535					1540					1545
Lys	Gln	Met	Glu	Asn	Ile	Leu	Tyr	Trp	Ile	Asn	Leu	Val	Phe	Val
				1550					1555					1560
Ile	Phe	Phe	Thr	Cys	Glu	Cys	Val	Leu	Lys	Met	Phe	Ala	Leu	Arg
				1565					1570					1575
His	Tyr	Tyr	Phe	Thr	Ile	Gly	Trp	Asn	Ile	Phe	Asp	Phe	Val	Val
				1580					1585					1590
Val	Ile	Leu	Ser	Ile	Val	Gly	Met	Phe	Leu	Ala	Asp	Ile	Ile	Glu
				1595					1600					1605
Lys	Tyr	Phe	Val	Ser	Pro	Thr	Leu	Phe	Arg	Val	Ile	Arg	Leu	Ala
				1610					1615					1620
Arg	Ile	Gly	Arg	Ile	Leu	Arg	Leu	Ile	Lys	Gly	Ala	Lys	Gly	Ile
				1625					1630					1635
Arg	Thr	Leu	Leu	Phe	Ala	Leu	Met	Met	Ser	Leu	Pro	Ala	Leu	Phe
				1640					1645					1650
Asn	Ile	Gly	Leu	Leu	Leu	Phe	Leu	Val	Met	Phe	Ile	Phe	Ser	Ile
				1655					1660					1665
Phe	Gly	Met	Ser	Asn	Phe	Ala	Tyr	Val	Lys	His	Glu	Ala	Gly	Ile
				1670					1675					1680
Asp	Asp	Met	Phe	Asn	Phe	Glu	Thr	Phe	Gly	Asn	Ser	Met	Ile	Cys
				1685					1690					1695
Leu	Phe	Gln	Ile	Thr	Thr	Ser	Ala	Gly	Trp	Asp	Gly	Leu	Leu	Leu
				1700					1705					1710
Pro	Ile	Leu	Asn	Arg	Pro	Pro	Asp	Cys	Ser	Leu	Asp	Lys	Glu	His
				1715					1720					1725
Pro	Gly	Ser	Gly	Phe	Lys	Gly	Asp	Cys	Gly	Asn	Pro	Ser	Val	Gly
				1730					1735					1740
Ile	Phe	Phe	Phe	Val	Ser	Tyr	Ile	Ile	Ile	Ser	Phe	Leu	Ile	Val
				1745					1750					1755

Val	Asn	Met	Tyr	Ile	Ala	Ile	Ile	Leu	Glu	Asn	Phe	Ser	Val	Ala
				1760					1765					1770
Thr	Glu	Glu	Ser	Ala	Asp	Pro	Leu	Ser	Glu	Asp	Asp	Phe	Glu	Thr
				1775					1780					1785
Phe	Tyr	Glu	Ile	Trp	Glu	Lys	Phe	Asp	Pro	Asp	Ala	Thr	Gln	Phe
				1790					1795					1800
Ile	Glu	Tyr	Cys	Lys	Leu	Ala	Asp	Phe	Ala	Asp	Ala	Leu	Glu	His
				1805					1810					1815
Pro	Leu	Arg	Val	Pro	Lys	Pro	Asn	Thr	Ile	Glu	Leu	Ile	Ala	Met
				1820					1825					1830
Asp	Leu	Pro	Met	Val	Ser	Gly	Asp	Arg	Ile	His	Cys	Leu	Asp	Ile
				1835					1840					1845
Leu	Phe	Ala	Phe	Thr	Lys	Arg	Val	Leu	Gly	Asp	Ser	Gly	Glu	Leu
				1850					1855					1860
Asp	Ile	Leu	Arg	Gln	Gln	Met	Glu	Glu	Arg	Phe	Val	Ala	Ser	Asn
				1865					1870					1875
Pro	Ser	Lys	Val	Ser	Tyr	Glu	Pro	Ile	Thr	Thr	Thr	Leu	Arg	Arg
				1880					1885					1890
Lys	Gln	Glu	Glu	Val	Ser	Ala	Val	Val	Leu	Gln	Arg	Ala	Tyr	Arg
				1895					1900					1905
Gly	His	Leu	Ala	Arg	Arg	Gly	Phe	Ile	Cys	Arg	Lys	Met	Ala	Ser
				1910					1915					1920
Asn	Lys	Leu	Glu	Asn	Gly	Gly	Thr	His	Arg	Asp	Lys	Lys	Glu	Ser
				1925					1930					1935
Thr	Pro	Ser	Thr	Ala	Ser	Leu	Pro	Ser	Tyr	Asp	Ser	Val	Thr	Lys
				1940					1945					1950
Pro	Asp	Lys	Glu	Lys	Gln	Gln	Arg	Ala	Glu	Glu	Gly	Arg	Arg	Glu
				1955					1960					1965
Arg	Ala	Lys	Arg	Gln	Lys	Glu	Val	Arg	Glu	Ser	Lys	Cys		
				1970					1975					

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1988 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(F) TISSUE TYPE: dorsal root ganglia

(G) CELL TYPE: peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Ala	Arg	Leu	Leu	Ala	Pro	Pro	Gly	Pro	Asp	Ser	Phe	Lys
				5					10					15
Pro	Phe	Thr	Pro	Glu	Ser	Leu	Ala	Asn	Ile	Glu	Arg	Arg	Ile	Ala
				20					25					30
Glu	Ser	Lys	Leu	Lys	Lys	Pro	Pro	Lys	Ala	Asp	Gly	Ser	His	Arg
				35					40					45
Glu	Asp	Asp	Glu	Asp	Ser	Lys	Pro	Lys	Pro	Asn	Ser	Asp	Leu	Glu
				50					55					60
Ala	Gly	Lys	Ser	Leu	Pro	Phe	Ile	Tyr	Gly	Asp	Ile	Pro	Gln	Gly
				65					70					75
Leu	Val	Ala	Val	Pro	Leu	Glu	Asp	Phe	Asp	Pro	Tyr	Tyr	Leu	Thr
				80					85					90
Gln	Lys	Thr	Phe	Val	Val	Leu	Asn	Arg	Gly	Lys	Thr	Leu	Phe	Arg
				95					100					105
Phe	Ser	Ala	Thr	Pro	Ala	Leu	Tyr	Ile	Leu	Ser	Pro	Phe	Asn	Leu
				110					115					120
Ile	Arg	Arg	Ile	Ala	Ile	Lys	Ile	Leu	Ile	His	Ser	Val	Phe	Ser
				125					130					135
Met	Ile	Ile	Met	Cys	Thr	Ile	Leu	Thr	Asn	Cys	Val	Phe	Met	Thr
				140					145					150
Phe	Ser	Asn	Pro	Pro	Glu	Trp	Ser	Lys	Asn	Val	Glu	Tyr	Thr	Phe
				155					160					165
Thr	Gly	Ile	Tyr	Thr	Phe	Glu	Ser	Leu	Val	Lys	Ile	Ile	Ala	Arg
				170					175					180
Gly	Phe	Cys	Ile	Asp	Gly	Phe	Thr	Phe	Leu	Arg	Asp	Pro	Trp	Asn
				185					190					195

Trp	Leu	Asp	Phe	Ser	Val	Ile	Met	Met	Ala	Tyr	Val	Thr	Glu	Phe
				200					205					210
Val	Asp	Leu	Gly	Asn	Val	Ser	Ala	Leu	Arg	Thr	Phe	Arg	Val	Leu
				215					220					225
Arg	Ala	Leu	Lys	Thr	Ile	Ser	Val	Ile	Pro	Gly	Leu	Lys	Thr	Ile
				230					235					240
Val	Gly	Ala	Leu	Ile	Gln	Ser	Val	Lys	Lys	Leu	Ser	Asp	Val	Met
				245					250					255
Ile	Leu	Thr	Val	Phe	Cys	Leu	Ser	Val	Phe	Ala	Leu	Ile	Gly	Leu
				260					265					270
Gln	Leu	Phe	Met	Gly	Asn	Leu	Arg	Asn	Lys	Cys	Val	Val	Trp	Pro
				275					280					285
Ile	Asn	Phe	Asn	Glu	Ser	Tyr	Leu	Glu	Asn	Gly	Thr	Arg	Gly	Phe
				290					295					300
Asp	Trp	Glu	Glu	Tyr	Ile	Asn	Asn	Lys	Thr	Asn	Phe	Tyr	Met	Val
				305					310					315
Pro	Gly	Met	Leu	Glu	Pro	Leu	Leu	Cys	Gly	Asn	Ser	Ser	Asp	Ala
				320					325					330
Gly	Gln	Cys	Pro	Glu	Gly	Phe	Gln	Cys	Met	Lys	Ala	Gly	Arg	Asn
				335					340					345
Pro	Asn	Tyr	Gly	Tyr	Thr	Ser	Phe	Asp	Thr	Phe	Ser	Trp	Ala	Phe
				350					355					360
Leu	Ala	Leu	Phe	Arg	Leu	Met	Thr	Gln	Asp	Tyr	Trp	Glu	Asn	Leu
				365					370					375
Tyr	Gln	Leu	Thr	Leu	Arg	Ala	Ala	Gly	Lys	Thr	Tyr	Met	Ile	Phe
				380					385					390
Phe	Val	Leu	Val	Ile	Phe	Val	Gly	Ser	Phe	Tyr	Leu	Val	Asn	Leu
				395					400					405
Ile	Leu	Ala	Val	Val	Ala	Met	Ala	Tyr	Glu	Glu	Gln	Asn	Gln	Ala
				410					415					420
Thr	Leu	Glu	Glu	Ala	Glu	Gln	Lys	Glu	Ala	Glu	Phe	Lys	Ala	Met
				425					430					435
Leu	Glu	Gln	Leu	Lys	Lys	Gln	Gln	Glu	Glu	Ala	Gln	Ala	Ala	Ala
				440					445					450
Met	Ala	Thr	Ser	Ala	Gly	Thr	Val	Ser	Glu	Asp	Ala	Ile	Glu	Glu
				455					460					465

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Glu Gly Glu Asp Gly Val Gly Ser Pro Arg Ser Ser Ser Glu Leu
                470                 475                 480
Ser Lys Leu Ser Ser Lys Ser Ala Lys Glu Arg Arg Asn Arg Arg
                485                 490                 495
Lys Lys Arg Lys Gln Lys Glu Leu Ser Glu Gly Glu Glu Lys Gly
                500                 505                 510
Asp Pro Glu Lys Val Phe Lys Ser Glu Ser Glu Asp Gly Met Arg
                515                 520                 525
Arg Lys Ala Phe Arg Leu Pro Asp Asn Arg Ile Gly Arg Lys Phe
                530                 535                 540
Ser Ile Met Asn Gln Ser Leu Leu Ser Ile Pro Gly Ser Pro Phe
                545                 550                 555
Leu Ser Arg His Asn Ser Lys Ser Ser Ile Phe Ser Phe Arg Gly
                560                 565                 570
Pro Gly Arg Phe Arg Asp Pro Gly Ser Glu Asn Glu Phe Ala Asp
                575                 580                 585
Asp Glu His Ser Thr Val Glu Glu Ser Glu Gly Arg Arg Asp Ser
                590                 595                 600
Leu Phe Ile Pro Ile Arg Ala Arg Glu Arg Arg Ser Ser Tyr Ser
                605                 610                 615
Gly Tyr Ser Gly Tyr Ser Gln Cys Ser Arg Ser Ser Arg Ile Phe
                620                 625                 630
Pro Ser Leu Arg Arg Ser Val Lys Arg Asn Ser Thr Val Asp Cys
                635                 640                 645
Asn Gly Val Val Ser Leu Ile Gly Pro Gly Ser His Ile Gly Arg
                650                 655                 660
Leu Leu Pro Glu Val Lys Ile Asp Lys Ala Ala Thr Asp Ser Ala
                665                 670                 675
Thr Thr Glu Val Glu Ile Lys Lys Lys Gly Pro Gly Ser Leu Leu
                680                 685                 690
Val Ser Met Asp Gln Leu Ala Ser Tyr Gly Arg Lys Asp Arg Ile
                695                 700                 705
Asn Ser Ile Met Ser Val Val Thr Asn Thr Leu Val Glu Glu Leu
                710                 715                 720
Glu Glu Ser Gln Arg Lys Cys Pro Pro Cys Trp Tyr Lys Phe Ala
                725                 730                 735
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Asn	Thr	Phe	Leu	Ile	Trp	Glu	Cys	His	Pro	Tyr	Trp	Ile	Lys	Leu
				740					745					750
Lys	Glu	Ile	Val	Asn	Leu	Ile	Val	Met	Asp	Pro	Phe	Val	Asp	Leu
				755					760					765
Ala	Ile	Thr	Ile	Cys	Ile	Val	Leu	Asn	Thr	Leu	Phe	Met	Ala	Met
				770					775					780
Glu	His	His	Pro	Met	Thr	Pro	Gln	Phe	Glu	His	Val	Leu	Ala	Val
				785					790					795
Gly	Asn	Leu	Val	Phe	Thr	Gly	Ile	Phe	Thr	Ala	Glu	Met	Phe	Leu
				800					805					810
Lys	Leu	Ile	Ala	Met	Asp	Pro	Tyr	Tyr	Tyr	Phe	Gln	Glu	Gly	Trp
				815					820					825
Asn	Ile	Phe	Asp	Gly	Phe	Ile	Val	Ser	Leu	Ser	Leu	Met	Glu	Leu
				830					835					840
Ser	Leu	Ala	Asp	Val	Glu	Gly	Leu	Ser	Val	Leu	Arg	Ser	Phe	Arg
				845					850					855
Leu	Leu	Arg	Val	Phe	Lys	Leu	Ala	Lys	Ser	Trp	Pro	Thr	Leu	Asn
				860					865					870
Met	Leu	Ile	Lys	Ile	Ile	Gly	Asn	Ser	Val	Gly	Ala	Leu	Gly	Asn
				875					880					885
Leu	Thr	Leu	Val	Leu	Ala	Ile	Ile	Val	Phe	Ile	Phe	Ala	Val	Val
				890					895					900
Gly	Met	Gln	Leu	Phe	Gly	Lys	Ser	Tyr	Lys	Glu	Cys	Val	Cys	Lys
				905					910					915
Ile	Asn	Gln	Glu	Cys	Lys	Leu	Pro	Arg	Trp	His	Met	Asn	Asp	Phe
				920					925					930
Phe	His	Ser	Phe	Leu	Ile	Val	Phe	Arg	Val	Leu	Cys	Gly	Glu	Trp
				935					940					945
Ile	Glu	Thr	Met	Trp	Asp	Cys	Met	Glu	Val	Ala	Gly	Gln	Ala	Met
				950					955					960
Cys	Leu	Ile	Val	Phe	Met	Met	Val	Met	Val	Ile	Gly	Asn	Leu	Val
				965					970					975
Val	Leu	Asn	Leu	Phe	Leu	Ala	Leu	Leu	Leu	Ser	Ser	Phe	Ser	Ala
				980					985					990
Asp	Asn	Leu	Ala	Ala	Thr	Asp	Asp	Asp	Gly	Glu	Met	Asn	Asn	Leu
				995					1000					1005

Gln	Ile	Ser	Val	Ile	Arg	Ile	Lys	Lys	Gly	Val	Ala	Trp	Thr	Lys
				1010					1015					1020
Val	Lys	Val	His	Ala	Phe	Met	Gln	Ala	His	Phe	Lys	Gln	Arg	Glu
				1025					1030					1035
Ala	Asp	Glu	Val	Lys	Pro	Leu	Asp	Glu	Leu	Tyr	Glu	Lys	Lys	Ala
				1040					1045					1050
Asn	Cys	Ile	Ala	Asn	His	Thr	Gly	Val	Asp	Ile	His	Arg	Asn	Gly
				1055					1060					1065
Asp	Phe	Gln	Lys	Asn	Gly	Asn	Gly	Thr	Thr	Ser	Gly	Ile	Gly	Ser
				1070					1075					1080
Ser	Val	Glu	Lys	Tyr	Ile	Ile	Asp	Glu	Asp	His	Met	Ser	Phe	Ile
				1085					1090					1095
Asn	Asn	Pro	Asn	Leu	Thr	Val	Arg	Val	Pro	Ile	Ala	Val	Gly	Glu
				1100					1105					1110
Ser	Asp	Phe	Glu	Asn	Leu	Asn	Thr	Glu	Asp	Val	Ser	Ser	Glu	Ser
				1115					1120					1125
Asp	Pro	Glu	Gly	Ser	Lys	Asp	Lys	Leu	Asp	Asp	Thr	Ser	Ser	Ser
				1130					1135					1140
Glu	Gly	Ser	Thr	Ile	Asp	Ile	Lys	Pro	Glu	Val	Glu	Glu	Val	Pro
				1145					1150					1155
Val	Glu	Gln	Pro	Glu	Glu	Tyr	Leu	Asp	Pro	Asp	Ala	Cys	Phe	Thr
				1160					1165					1170
Glu	Gly	Cys	Val	Gln	Arg	Phe	Lys	Cys	Cys	Gln	Val	Asn	Ile	Glu
				1175					1180					1185
Glu	Gly	Leu	Gly	Lys	Ser	Trp	Trp	Ile	Leu	Arg	Lys	Thr	Cys	Phe
				1190					1195					1200
Leu	Ile	Val	Glu	His	Asn	Trp	Phe	Glu	Thr	Phe	Ile	Ile	Phe	Met
				1205					1210					1215
Ile	Leu	Leu	Ser	Ser	Gly	Ala	Leu	Ala	Phe	Glu	Asp	Ile	Tyr	Ile
				1220					1225					1230
Glu	Gln	Arg	Lys	Thr	Ile	Arg	Thr	Ile	Leu	Glu	Tyr	Ala	Asp	Lys
				1235					1240					1245
Val	Phe	Thr	Tyr	Ile	Phe	Ile	Leu	Glu	Met	Leu	Leu	Lys	Trp	Thr
				1250					1255					1260
Ala	Tyr	Gly	Phe	Val	Lys	Phe	Phe	Thr	Asn	Ala	Trp	Cys	Trp	Leu
				1265					1270					1275

Asp Phe Leu Ile Val Ala Val Ser Leu Val Ser Leu Ile Ala Asn
1280 1285 1290

Ala Leu Gly Tyr Ser Glu Leu Gly Ala Ile Lys Ser Leu Arg Thr
1295 1300 1305

Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser Arg Phe Glu Gly
1310 1315 1320

Met Arg Val Val Val Asn Ala Leu Val Gly Ala Ile Pro Ser Ile
1325 1330 1335

Met Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Ile Phe Ser
1340 1345 1350

Ile Met Gly Val Asn Leu Phe Ala Gly Lys Tyr His Tyr Cys Phe
1355 1360 1365

Asn Glu Thr Ser Glu Ile Arg Phe Glu Ile Asp Ile Val Asn Asn
1370 1375 1380

Lys Thr Asp Cys Glu Lys Leu Met Glu Gly Asn Ser Thr Glu Ile
1385 1390 1395

Arg Trp Lys Asn Val Lys Ile Asn Phe Asp Asn Val Gly Ala Gly
1400 1405 1410

Tyr Leu Ala Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp
1415 1420 1425

Ile Met Tyr Ala Ala Val Asp Ser Arg Lys Pro Asp Glu Gln Pro
1430 1435 1440

Asp Tyr Glu Gly Asn Ile Tyr Met Tyr Ile Tyr Phe Val Ile Phe
1445 1450 1455

Ile Ile Phe Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile Gly Val
1460 1465 1470

Ile Ile Asp Asn Phe Asn Gln Gln Lys Lys Lys Phe Gly Gly Gln
1475 1480 1485

Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met
1490 1495 1500

Lys Lys Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro
1505 1510 1515

Leu Asn Lys Ile Gln Gly Ile Val Phe Asp Phe Val Thr Gln Gln
1520 1525 1530

Ala Phe Asp Ile Val Ile Met Met Leu Ile Cys Leu Asn Met Val
1535 1540 1545

Thr	Met	Met	Val	Glu	Thr	Asp	Thr	Gln	Ser	Lys	Gln	Met	Glu	Asn
				1550					1555					1560
Ile	Leu	Tyr	Trp	Ile	Asn	Leu	Val	Phe	Val	Ile	Phe	Phe	Thr	Cys
				1565					1570					1575
Glu	Cys	Val	Leu	Lys	Met	Phe	Ala	Leu	Arg	His	Tyr	Tyr	Phe	Thr
				1580					1585					1590
Ile	Gly	Trp	Asn	Ile	Phe	Asp	Phe	Val	Val	Val	Ile	Leu	Ser	Ile
				1595					1600					1605
Val	Gly	Met	Phe	Leu	Ala	Asp	Ile	Ile	Glu	Lys	Tyr	Phe	Val	Ser
				1610					1615					1620
Pro	Thr	Leu	Phe	Arg	Val	Ile	Arg	Leu	Ala	Arg	Ile	Gly	Arg	Ile
				1625					1630					1635
Leu	Arg	Leu	Ile	Lys	Gly	Ala	Lys	Gly	Ile	Arg	Thr	Leu	Leu	Phe
				1640					1645					1650
Ala	Leu	Met	Met	Ser	Leu	Pro	Ala	Leu	Phe	Asn	Ile	Gly	Leu	Leu
				1655					1660					1665
Leu	Phe	Leu	Val	Met	Phe	Ile	Phe	Ser	Ile	Phe	Gly	Met	Ser	Asn
				1670					1675					1680
Phe	Ala	Tyr	Val	Lys	His	Glu	Ala	Gly	Ile	Asp	Asp	Met	Phe	Asn
				1685					1690					1695
Phe	Glu	Thr	Phe	Gly	Asn	Ser	Met	Ile	Cys	Leu	Phe	Gln	Ile	Thr
				1700					1705					1710
Thr	Ser	Ala	Gly	Trp	Asp	Gly	Leu	Leu	Leu	Pro	Ile	Leu	Asn	Arg
				1715					1720					1725
Pro	Pro	Asp	Cys	Ser	Leu	Asp	Lys	Glu	His	Pro	Gly	Ser	Gly	Phe
				1730					1735					1740
Lys	Gly	Asp	Cys	Gly	Asn	Pro	Ser	Val	Gly	Ile	Phe	Phe	Phe	Val
				1745					1750					1755
Ser	Tyr	Ile	Ile	Ile	Ser	Phe	Leu	Ile	Val	Val	Asn	Met	Tyr	Ile
				1760					1765					1770
Ala	Ile	Ile	Leu	Glu	Asn	Phe	Ser	Val	Ala	Thr	Glu	Glu	Ser	Ala
				1775					1780					1785
Asp	Pro	Leu	Ser	Glu	Asp	Asp	Phe	Glu	Thr	Phe	Tyr	Glu	Ile	Trp
				1790					1795					1800
Glu	Lys	Phe	Asp	Pro	Asp	Ala	Thr	Gln	Phe	Ile	Glu	Tyr	Cys	Lys
				1805					1810					1815

Leu Ala Asp Phe Ala Asp Ala Leu Glu His Pro Leu Arg Val Pro
1820 1825 1830

Lys Pro Asn Thr Ile Glu Leu Ile Ala Met Asp Leu Pro Met Val
1935 1840 1845

Ser Gly Asp Arg Ile His Cys Leu Asp Ile Leu Phe Ala Phe Thr
1850 1855 1860

Lys Arg Val Leu Gly Asp Ser Gly Glu Leu Asp Ile Leu Arg Gln
1865 1870 1875

Gln Met Glu Glu Arg Phe Val Ala Ser Asn Pro Ser Lys Val Ser
1880 1885 1890

Tyr Glu Pro Ile Thr Thr Thr Leu Arg Arg Lys Gln Glu Glu Val
1895 1900 1905

Ser Ala Val Val Leu Gln Arg Ala Tyr Arg Gly His Leu Ala Arg
1910 1915 1920

Arg Gly Phe Ile Cys Arg Lys Met Ala Ser Asn Lys Leu Glu Asn
1925 1930 1935

Gly Gly Thr His Arg Asp Lys Lys Glu Ser Thr Pro Ser Thr Ala
1940 1945 1950

Ser Leu Pro Ser Tyr Asp Ser Val Thr Lys Pro Asp Lys Glu Lys
1955 1960 1965

Gln Gln Arg Ala Glu Glu Gly Arg Arg Glu Arg Ala Lys Arg Gln
1970 1975 1980

Lys Glu Val Arg Glu Ser Lys Cys
1985

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 696 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RT-PCR

(A) DESCRIPTION: /desc = „DNA probe“

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(F) TISSUE TYPE: dorsal root ganglia

(G) CELL TYPE: peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
  1 CTCAACATGG TTACTATGAT GGTGGAGACA GACACTCAGA GCAAGCAGAT
 51 GGAGAACATT CTTTACTGGA TTAATCTGGT CTTTGTCATC TTCTTCACCT
101 GCGAGTGTGT GCTCAAAATG TTTGCCTTGA GACACTACTA TTTCACCATT
151 GGCTGGAACA TCTTTGACTT TGTGGTGGTC ATCCTCTCCA TTGTGGGAAT
201 GTTCCTGGCT GATATCATTG AGAAGTACTT CGTCTCCCCA ACCCTATTCC
251 GAGTTATCCG ATTGGCCCGT ATTGGGCGCA TCTTGCGTCT GATCAAGGGG
301 GCCAAAGGGA TCCGCACCCT GCTCTTTGGC CTTAATGATG TCGCTGGCCG
351 CCCTGTTCAA CATCGCCTCC TGCTCTTCCT CGTCATGTTC ATCTTCTCCA
401 TTTTTGGCAT GTCCAACTTC GCATACGTGA AGCACGAGGC CGGCATTGAC
451 GACATGTTCA ACTTCGAGAC ATTTGGCAAC AGCATGATCT GTTTGTTCCA
501 GATCACAACG TCTGCTGGCT GGGATGGCCT GCTGCTGCCA ATCCTGAACC
551 GCCCCCCTGA CTGCAGCTTG GACAAAGAGC ACCCAGGGAG TGGCTTCAAA
601 GGGGACTGTG GGAACCCCTC GGTGGGCATC TTCTTCTTTG TGAGCTACAT
651 CATCATCTCC TTCCTGATTG TGGTGAACAT GTACATCGCA GTCATC
```

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 232 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(F) TISSUE TYPE: dorsal root ganglia

(G) CELL TYPE: peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu	Asn	Met	Val	Thr	Met	Met	Val	Glu	Thr	Asp	Thr	Gln	Ser	Lys
				5					10					15
Gln	Met	Glu	Asn	Ile	Leu	Tyr	Trp	Ile	Asn	Leu	Val	Phe	Val	Ile
				20					25					30
Phe	Phe	Thr	Cys	Glu	Cys	Val	Leu	Lys	Met	Phe	Ala	Leu	Arg	His
				35					40					45
Tyr	Tyr	Phe	Thr	Ile	Gly	Trp	Asn	Ile	Phe	Asp	Phe	Val	Val	Val
				50					55					60
Ile	Leu	Ser	Ile	Val	Gly	Met	Phe	Leu	Ala	Asp	Ile	Ile	Glu	Lys
				65					70					75
Tyr	Phe	Val	Ser	Pro	Thr	Leu	Phe	Arg	Val	Ile	Arg	Leu	Ala	Arg
				80					85					90
Ile	Gly	Arg	Ile	Leu	Arg	Leu	Ile	Lys	Gly	Ala	Lys	Gly	Ile	Arg
				95					100					105
Thr	Leu	Leu	Phe	Gly	Leu	Asn	Asp	Val	Ala	Gly	Arg	Pro	Val	Gln
				110					115					120
His	Arg	Leu	Leu	Leu	Phe	Leu	Val	Met	Phe	Ile	Phe	Ser	Ile	Phe
				125					130					135
Gly	Met	Ser	Asn	Phe	Ala	Tyr	Val	Lys	His	Glu	Ala	Gly	Ile	Asp
				140					145					150
Asp	Met	Phe	Asn	Phe	Glu	Thr	Phe	Gly	Asn	Ser	Met	Ile	Cys	Leu
				155					160					165

Phe	Gln	Ile	Thr	Thr	Ser	Ala	Gly	Trp	Asp	Gly	Leu	Leu	Leu	Pro
				170					175					180
Ile	Leu	Asn	Arg	Pro	Pro	Asp	Cys	Ser	Leu	Asp	Lys	Glu	His	Pro
				185					190					195
Gly	Ser	Gly	Phe	Lys	Gly	Asp	Cys	Gly	Asn	Pro	Ser	Val	Gly	Ile
				200					205					210
Phe	Phe	Phe	Val	Ser	Tyr	Ile	Ile	Ile	Ser	Phe	Leu	Ile	Val	Val
				215					220					225
Asn	Met	Tyr	Ile	Ala	Val	Ile								
				230										

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6556 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(F) TISSUE TYPE: Dorsal root ganglia

(G) CELL TYPE: Peripheral nerve

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CCAAGATGGC GCCCACCGCA GTCCCGCCCG CCGCAGCCTC GGCGCCTCTG    50
CAGTCCGGCC GCGCCTCCCG GGCCCCGCGC TAGGGCCGCT GCCGCCTCGC   100
CCGCCGCCGC CGCCGCCAGC TGACCTGTCC CGGACACATA ACTAACGAAG   150
CTGCTGCAGG ATGAGAAGAT GGCAGCGCGG CTGCTCGCAC CACCAGGCCC   200
TGATAGTTTC AAGCCTTTCA CCCCTGAGTC GCTGGCAAAC ATCGAGAGGC   250
GTATTGCCGA GAGCAAGCTC AAGAAACCAC CAAAGGCGGA TGGCAGCCAC   300
CGGGAGGACG ATGAAGACAG CAAGCCCAAG CCAAACAGTG ACCTGGAGGC   350
TGGGAAGAGT TTGCCTTTCA TCTACGGGGA CATCCCGCAA GGCCTGGTTG   400
CGGTTCCCCT GGAGGACTTT GACCCTTACT ATTTGACGCA GAAAACCTTT   450
GTAGTATTAA ACAGAGGGAA AACTCTCTTC AGATTTAGTG CCACACCTGC   500
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CTTGTACATT TTAAGCCCTT TTAACCTGAT AAGAAGAATA GCTATTAAAA 550
TTTTGATACA CTCAGTTTTC AGCATGATCA TCATGTGCAC CATCCTGACC 600
AACTGTGTGT TCATGACCTT TAGTAACCCT CCAGAATGGT CCAAGAATGT 650
GGAGTACACA TTCACAGGGA TTTACACATT TGAATCACTA GTGAAAATCA 700
TCGCAAGAGG TTTCTGCATA GACGGCTTCA CCTTCTTGCG AGACCCGTGG 750
AACTGGTTAG ACTTCAGTGT CATCATGATG GCATATGTGA CAGAGTTTGT 800
GGACCTGGGC AATGTCTCAG CGCTGAGAAC ATTCAGGGTT CTCCGAGCTT 850
TGAAAACTAT CTCTGTAATT CCAGGCCTGA AGACAATCGT GGGCGCCCTA 900
ATCCAGTCCG TGAAGAAGCT GTCGGACGTG ATGATCCTGA CAGTGTTCTG 950
CCTGAGTGTT TTCGCCCTGA TTGGCCTGCA GCTCTTCATG GGGAACCTT- 999
CGAAACAAGT GTGTCGTGTG GCCCATAAAC TTCAACGAGA GCTACCTGGA 1049
GAACGGCACC AGAGGCTTTG ACTGGGAGGA ATATATCAAC AATAAAACAA 1099
ACTTTTACAT GGTTCCTGGC ATGCTAGAAC CCTTGCTCTG CGGGAACAGT 1149
TCTGATGCTG GGCAATGCCC AGAGGGATTC CAGTGCATGA AAGCAGGAAG 1199
GAACCCCAAC TACGGTTACA CCAGCTTTGA CACCTTCAGC TGGGCCTTCT 1249
TGGCATTATT CCGCCTTATG ACCCAGGACT ATTGGGAGAA CTTATACCAG 1299
CTGACCTTAC GAGCCGCTGG GAAAACGTAC ATGATCTTCT TTGTCTTGGT 1349
CATCTTCGTG GGTTCTTTCT ATCTGGTGAA CTTGATCTTG GCTGTGGTGG 1399
CCATGGCTTA TGAGGAACAG AACCAGGCAA CACTGGAGGA GGCAGAGCAA 1449
AAAGAGGCCG AGTTCAAGGC AATGCTGGAG CAACTCAAGA AGCAGCAGGA 1499
GGAGGCACAG GCTGCTGCAA TGGCCACCTC AGCGGGCACT GTCTCGGAAG 1549
ACGCCATTGA AGAAGAAGGG GAAGATGGGG TAGGCTCTCC GAGGAGCTCT 1599
TCTGAACTGT CTAAACTCAG TTCCAAGAGC GCGAAGGAGC GGCGGAACCG 1649
ACGGAAGAAG AGGAAGCAGA AGGAGCTCTC TGAAGGCGAG GAGAAAGGGG 1699
ACCCGGAGAA GGTGTTTAAG TCAGAGTCGG AAGACGGTAT GAGAAGGAAG 1749
GCCTTCCGGC TGCCAGACAA CAGGATAGGG AGGAAGTTTT CCATCATGAA 1799
TCAGTCGCTG CTCAGCATTC CAGGCTCGCC CTTCCTCTCC CGACATAACA 1849
GCAAAAGCAG CATCTTCAGC TTCCGGGGAC CCGGTCGGTT CCGGGACCCC 1899
GGCTCTGAGA ATGAGTTCGC AGACGATGAA CACAGCACCG TGGAGGAGAG 1949
CGAGGGCCGG CGTGACTCGC TCTTCATCCC GATCCGCGCC CGCGAGCGCC 1999
GCAGCAGCTA CAGTGGCTAC AGCGGCTACA GCCAGTGCAG CCGCTCGTCG 2049
CGCATCTTCC CCAGCCTGCG GCGCAGCGTG AAGCGCAACA GCACGGTGGA 2099
CTGCAACGGC GTAGTGTCAC TCATCGGGCC CGGCTCACAC ATCGGGCGGC 2149
TCCTGCCTGA GGCAACGACT GAGGTGGAAA TTAAGAAGAA AGGCCCTGGA 2199
TCTCTTTTAG TTTCTATGGA CCAACTCGCC TCCTACGGAC GGAAGGACAG 2249
AATCAACAGC ATAATGAGCG TGGTCACAAA CACGCTAGTG GAAGAGCTGG 2299

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AAGAGTCTCA GAGAAAGTGC CCACCGTGCT GGTATAAGTT TGCCAACACT 2349
TTCCTCATCT GGGAGTGTCA CCCCTACTGG ATAAAACTGA AGGAGATCGT 2399
GAACTTAATC GTCATGGACC CTTTTGTAGA CTTAGCCATC ACCATCTGCA 2449
TCGTTCTGAA TACGCTATTT ATGGCAATGG AGCACCATCC CATGACACCA 2499
CAGTTCGAAC ACGTCTTGGC CGTAGGAAAT CTGGTGTTCA CCGGGATCTT 2549
CACGGCGGAA ATGTTTCTGA AGCTCATAGC CATGGACCCC TACTATTATT 2599
TCCAAGAAGG CTGGAACATT TTTGACGGAT TTATTGTCTC CCTCAGTTTA 2649
ATGGAGCTGA GTCTCGCAGA TGTGGAGGGG CTCTCAGTGC TGCGGTCTTT 2699
CCGACTGCTC CGAGTCTTCA AGCTGGCCAA GTCCTGGCCC ACCCTGAACA 2749
TGCTGATCAA GATCATCGGG AACTCCGTGG GTGCCCTGGG CAACCTGACC 2799
CTGGTGCTGG CCATCATCGT CTTCATCTTC GCCGTGGTGG GGATGCAGCT 2849
GTTTGGAAAG AGTTACAAGG AGTGCGTCTG TAAGATCAAC CAGGAGTGCA 2899
AGCTCCCGCG CTGGCACATG AACGACTTCT TCCACTCCTT CCTCATCGTC 2949
TTCCGAGTGC TGTGTGGGGA GTGGATCGAG ACCATGTGGG ACTGCATGGA 2999
GGTGGCCGGC CAGGCCATGT GCCTCATTGT CTTCATGATG GTTATGGTCA 3049
TTGGCAACCT GGTGGTGCTG AATCTATTCC TGGCCTTGCT TCTGAGCTCC 3099
TTCAGCGCAG ACAACCTGGC GGCCACAGAC GACGACGGGG AAATGAACAA 3149
CCTGCAGATC TCAGTGATCC GGATCAAGAA GGGCGTGGCC TGGACCAAAG 3199
TGAAGGTGCA CGCCTTCATG CAGGCTCACT TCAAGCAGCG GGAGGCGGAT 3249
GAAGTGAAAC CCCTCGACGA GCTGTATGAG AAGAAGGCCA ACTGCATCGC 3299
CAACCACACG GGCGTGGATA TCCACCGGAA CGGCGACTTC CAGAAGAACG 3349
GGAACGGAAC CACCAGCGGC ATCGGCAGCA GCGTGGAGAA GTACATCATC 3399
GACGAGGACC ACATGTCCTT CATTAACAAC CCAAACCTGA CCGTCCGGGT 3449
GCCCATTGCT GTGGGCGAGT CTGACTTCGA GAACCTCAAC ACAGAGGATG 3499
TTAGCAGCGA ATCAGACCCT GAAGGCAGCA AAGATAAACT GGACGATACC 3549
AGCTCCTCAG AAGGAAGTAC CATCGACATC AAGCCTGAGG TGGAAGAAGT 3599
TCCCGTGGAG CAACCTGAGG AATACTTGGA TCCGGACGCC TGCTTTACAG 3649
AGGGTTGCGT CCAGCGGTTC AAGTGCTGCC AGGTCAACAT CGAGGAAGGA 3699
CTAGGCAAGT CGTGGTGGAT CTTGCGGAAA ACCTGCTTCC TCATTGTGGA 3749
GCACAATTGG TTTGAGACCT TCATCATCTT CATGATTCTG CTCAGCAGTG 3799
GCGCCCTGGC CTTTGAGGAC ATCTACATTG AGCAGAGGAA GACCATCCGC 3849
ACCATCCTGG AGTATGCGGA CAAGGTCTTC ACCTACATCT TCATCCTGGA 3899
GATGTTGCTC AAGTGGACAG CCTACGGCTT CGTCAAGTTC TTCACCAATG 3949
CCTGGTGCTG GTTGGACTTC CTCATTGTGG CTGTCTCTTT AGTCAGCCTT 3999
ATAGCTAATG CCCTGGGCTA CTCGGAACTA GGTGCCATAA AGTCCCTTAG 4049
GACCCTAAGA GCTTTGAGAC CCTTAAGAGC CTTATCACGA TTTGAAGGGA 4099
```

```
TGAGGGTGGT GGTGAATGCC TTGGTGGGCG CCATCCCCTC CATCATGAAT 4149
GTGCTGCTGG TGTGTCTCAT CTTCTGGCTG ATTTTCAGCA TCATGGGAGT 4199
TAACCTGTTT GCGGGGAAAT ACCACTACTG CTTTAATGAG ACTTCTGAAA 4249
TCCGGTTCGA AATCGATATT GTCAACAATA AAACGGACTG TGAGAAGCTC 4299
ATGGAGGGCA ACAGCACGGA GATCCGATGG AAGAATGTCA AGATCAACTT 4349
TGACAATGTC GGAGCAGGGT ACCTGGCCCT TCTTCAAGTG GCAACCTTCA 4399
AAGGCTGGAT GGACATCATG TATGCGGCTG TAGATTCCCG AAAGCCAGAC 4449
GAGCAGCCTG ACTACGAGGG CAACATCTAC ATGTACATCT ACTTCGTCAT 4499
CTTCATCATC TTCGGCTCCT TCTTCACCCT CAACCTGTTC ATCGGTGTCA 4549
TCATCGACAA CTTCAACCAG CAGAAGAAAA AGTTTGGAGG TCAGGACATC 4599
TTCATGACAG AGGAACAGAA GAAGTACTAC AATGCCATGA AAAAGCTGGG 4649
CTCCAAGAAG CCACAGAAGC CCATCCCCCG ACCCTTGAAC AAAATCCAAG 4699
GGATTGTCTT TGATTTCGTC ACTCAACAAG CCTTTGACAT TGTGATCATG 4749
ATGCTCATCT GCCTTAACAT GGTGACAATG ATGGTGGAGA CAGACACTCA 4799
GAGCAAGCAG ATGGAGAACA TTCTTTACTG GATTAATCTG GTCTTTGTCA 4849
TCTTCTTCAC CTGCGAGTGT GTGCTCAAAA TGTTTGCCTT GAGACACTAC 4899
TATTTCACCA TTGGCTGGAA CATCTTTGAC TTTGTGGTGG TCATCCTCTC 4949
CATTGTGGGA ATGTTCCTGG CTGATATCAT TGAGAAGTAC TTCGTCTCCC 4999
CAACCCTATT CCGAGTTATC CGATTGGCCC GTATTGGGCG CATCTTGCGT 5049
CTGATCAAGG GCGCCAAAGG GATCCGCACC CTGCTCTTTG CCTTAATGAT 5099
GTCGCTGCCC GCCCTGTTCA ACATCGGCCT CCTGCTCTTC CTCGTCATGT 5149
TCATCTTCTC CATTTTTGGC ATGTCCAACT TCGCATACGT GAAGCACGAG 5199
GCCGGCATTG ACGACATGTT CAACTTCGAG ACATTTGGCA ACAGCATGAT 5249
CTGTTTGTTC CAGATCACAA CGTCTGCTGG CTGGGATGGC CTGCTGCTGC 5299
CAATCCTGAA CCGCCCCCCT GACTGCAGCT TGGACAAAGA GCACCCAGGG 5349
AGTGGCTTCA AAGGGGACTG TGGGAACCCC TCGGTGGGCA TCTTCTTCTT 5399
TGTGAGCTAC ATCATCATCT CCTTCCTGAT TGTGGTGAAC ATGTACATCG 5449
CCATCATCCT GGAGAACTTC AGCGTGGCCA CCGAGGAGAG CGCCGACCCT 5499
CTGAGTGAGG ATGACTTCGA GACTTTCTAT GAGATCTGGG AGAAGTTTGA 5549
CCCAGACGCC ACCCAGTTCA TCGAGTACTG TAAGCTGGCA GACTTTGCCG 5599
ACGCCCTGGA GCACCCGCTC CGAGTACCCA AGCCCAACAC CATCGAGCTC 5649
ATCGCCATGG ACCTGCCCAT GGTGAGCGGA GATCGCATCC ACTGCTTGGA 5699
CATCCTTTTC GCCTTCACCA AGCGAGTCCT GGGAGACAGT GGGGAGTTGG 5749
ACATCCTGCG GCAGCAGATG GAGGAGCGGT TCGTGGCATC CAATCCTTCC 5799
AAAGTGTCTT ACGA-GCCTA TCACAACCAC TCTGCGGCGC AAGCAGGAGG 5848
AGGTGTCTGC AGTGGTCCTG CAGCGTGCCT ACAGGGGACA CTTGGCTAGG 5898
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CGGGGCTTCA	TCTGCAGAAA	GATGGCCTCC	AACAAGCTGG	AGAATGGAGG	5948
CACACACAGA	GACAAGAAGG	AGAGCACCCC	GTCCACAGCC	TCCCTCCCCT	5998
CTTACGACAG	CGTCACAAAG	CCAGACAAGG	AGAAGCAGCA	GCGTGCGGAG	6048
GAGGGCAGAA	GGGAAAGAGC	CAAGAGGCAA	AAAGAGGTCA	GGGAGTCCAA	6098
GTGCTAGAGG	AGGGGAAAGG	AAGCTTACCC	CGGCTGAACA	CTGGCAAGTG	6148
AAAGCTTGTT	TACAAACTTC	CGAATCTCAC	GGATGCAGAG	CAGCTGTGCA	6198
GACGCTCGCT	GTACTGGAAG	ACCTATACCA	AACATAGTCT	GCTTACATGT	6248
GACATGGTGG	CATCCTGAGC	GGTGA---CT	GCTGGGGACA	AAGGACCCTG	6295
CTCCCTGGAC	TCACAGATCT	CCTATCGCTT	GGGCAGACGG	TTACTGCATG	6345
TTCCACACTT	AGTCAATGCA	ACTTAGGACT	AAACTAACCA	GGATACAAAA	6395
CCGAGGCGGC	TGCCGGGACC	AGCAGATCAC	CGCTGCAGCC	AAATGGATTT	6445
TATTTTTTCA	TTTTGTTGAT	TCTCAGAAGC	AGAAAGCATC	ACTTTAAAAG	6495
TTTGTTTGTT	CATNCAAACA	ATATTTGAAT	TCTTACATTA	GTTAAGCTAA	6545
GCANCAAAAA	G---------	----------	----------	----------	6556

Claims

1. An isolated DNA sequence comprising the nucleotide sequence set forth in SEQ ID NO:7.

2. The isolated DNA sequence of claim 1 comprising the nucleotide sequence as set forth in SEQ ID NO:1.

3. The DNA of Claim 2 wherein said DNA sequence is encoding a sodium channel protein.

4. The DNA of Claim 3 wherein said sodium channel protein is the α-subunit.

5. The DNA of Claim 4 wherein said sodium channel protein is tetrodotoxin-sensitive.

6. The DNA of Claim 5 wherein said sodium channel protein is found in mammals.

7. The DNA of Claim 5 wherein said sodium channel protein is found in rat.

8. The DNA of Claim 5 wherein said sodium channel protein is found in human.

9. The DNA of Claim 2 wherein said DNA is cDNA.

10. The DNA of Claim 2 wherein said DNA is synthetic DNA.

11. Expression vectors comprising a DNA as claimed in claims 2 to 10.

12. Host cells transformed with an expression vector of Claim 11.

13. A cDNA library comprising a host cell of Claim 12.

14. A recombinant polynucleotide comprising a nucleic acid sequence derived from a DNA sequence as claimed in claims 2 to 10.

15. A tetrodotoxin-sensitive sodium channel protein encoded by a DNA of claims 2 to 10 or allelic variants thereof.

16. The protein having the amino acid sequence set forth in SEQ ID NO:3.

17. An assay for inhibitors of tetrodotoxin-sensitive sodium channel protein encoded by a DNA of claims 2 to 10 or the amino acid sequence as set forth in SEQ ID NO:3 comprising contacting a compound suspected of being said inhibitor with expressed sodium channel protein and measuring the activity of said expressed sodium channel protein.

18. An isolated DNA sequence comprising the nucleotide sequence set forth in SEQ ID NO:7 having a 30 base pair insert after base pair number 2050, the 30 base pair insert comprising GTGAAAATAGATAAGGCAGCTACGGACAGC.

19. The isolated DNA sequence of claim 18 comprising the nucleotide sequence as set forth in SEQ ID NO:2.

20. The DNA of Claim 19 wherein said DNA sequence is encoding a sodium channel protein.

21. The DNA of Claim 20 wherein said sodium channel protein is the α-subunit.

22. The DNA of Claim 21 wherein said sodium channel protein is tetrodotoxin-sensitive.

23. The DNA of Claim 22 wherein said sodium channel protein is found in mammal.

24. The DNA of Claim 22 wherein said sodium channel protein is found in rat.

25. The DNA of Claim 22 wherein said sodium channel protein is found in human.

26. The DNA of Claim 19 wherein said DNA is cDNA.

27. The DNA of Claim 19 wherein said DNA is synthetic DNA.

28. Expression vectors comprising a DNA as claimed in claims 19 to 27.

29. Host cells transformed with an expression vector of Claim 28.

30. A cDNA library comprising a host cell of Claim 29.

31. A recombinant polynucleotide comprising a nucleic acid sequence derived from a DNA sequence as claimed in claims 19 to 27.

32. A tetrodotoxin-sensitive sodium channel protein encoded by a DNA of Claims 19 to 27 or allelic variants thereof.

33. The protein having the amino acid sequence set forth in SEQ ID NO:4.

34. An assay for inhibitors of tetrodotoxin-sensitive sodium channel protein encoded by a DNA of claims 19 to 27 or the amino acid sequence as set forth in SEQ ID NO:4 comprising contacting a compound suspected of being said inhibitor with expressed sodium channel protein and measuring the activity of said expressed sodium channel protein.

35. An isolated polynucleotide probe comprising the nucleotide sequence set forth in SEQ ID NO:5 and complements thereof.

36. The protein having the amino acid sequence set forth in SEQ ID NO:6.

37. A polynucleotide probe comprising the polynucleotide of Claim 35 bound to a reporter molecule.

38. A method of growing plasmids containing constructs of tetrodotexin-sensitive sodium channel proteins comprising:

employing competent *E. coli* cell lines for primary transformations which allow the stable propagation of plasmids containing unstable inserts following ligation reactions;

employing 1/2 strength freezing media mixed with a broth chosen from the group comprising full strength YENB, full strength YET, and full strength LB, the broth being mixed with agar for solid media;

employing full strength freezing media plus 1/2 strength LB for liquid media;

employing carbenicillin as an antibiotic;

providing a temperature no greater than 30° C; and

growing the plasmids for periods longer than 20 hours.

39. The method according to claim 38 employing competent *E. coli* cells for secondary transformants.

40. The method according to claim 38 wherein the temperature is 28° C.

41. The method according to claim 38 wherein the growing period is in the range of approximately 36 to 48 hours.

42. The method according to claim 38 having a carbenicillin concentration within the range of 50-200 μg/ml.

43. The method according to claim 38 wherein the carbenicillin has a concentration within the range of 75-125 μg/ml.

44. The method according to claim 38 wherein the carbenicillin has a concentration of 100 μg/ml.

45. Antibodies against a tetrodotoxin-sensitive sodium channel protein as claimed in claims 15, 16, 32 and 33.

46. The use of a tetrodotoxin-sensitive sodium channel protein as claimed in claims 15, 16, 32 and 33 for identifying inhibitors of their activity.

47. A method of producing a tetrodotoxin-sensitive sodium channel protein as claimed in claims 15, 16, 32 and 33, comprising cultivating a host cell as claimed in claim 12 or 29 in a suitable medium and optionally isolating said channel protein.

48. Proteins as claimed in claims 15, 16, 32 and 33 prepared by the method of claim 47.

49. Proteins as claimed in claims 15, 16, 32 and 33 for identifying inhibitors of their activity.

50. The invention substantially as hereinbefore described, especially with reference to the examples.

***

Fig. 1A: SEQ ID NO:3

```
  1  MAARLLAPPG PDSFKPFTPE SLANIERRIA ESKLKKPPKA DGSHREDDED

 51  SKPKPNSDLE AGKSLPFIYG DIPQGLVAVP LEDFDPYYLT QKTFVVLNRG

101  KTLFRFSATP ALYILSPFNL IRRIAIKILI HSVFSMIIMC TILTNCVFMT
                                     |-----------IS1-----------|
151  FSNPPEWSKN VEYTFTGIYT FESLVKIIAR GFCIDGFTFL RDPWNWLDFS
                |--------IS2---------|                |-------
201  VIMMAYVTEF VDLGNVSALR TFRVLRALKT ISVIPGLKTI VGALIQSVKK
     IS3--------|   ◆   |--------IS4---------|
251  LSDVMILTVF CLSVFALIGL QLFMGNLRNK CVVWPINFNE SYLENGTRGF
      •|-----------IS5-----------|                ◆     ◆
301  DWEEYINNKT NFYMVPGMLE PLLCGNSSDA GQCPEGFQCM KAGRNPNYGY
              ◆                 ◆
351  TSFDTFSWAF LALFRLMTQD YWENLYQLTL RAAGKTYMIF FVLVIFVGSF
                            Δ                |---------IS6-
401  YLVNLILAVV AMAYEEQNQA TLEEAEQKEA EFKAMLEQLK KQQEEAQAAA
     ------------|
451  MATSAGTVSE DAIEEEGEDG VGSPRSSSEL SKLSSKSAKE RRNRRKKRKQ

501  KELSEGEEKG DPEKVFKSES EDGMRRKAFR LPDNRIGRKF SIMNQSLLSI
                                                •    ◆
551  PGSPFLSRHN SKSSIFSFRG PGRFRDPGSE NEFADDEHST VEESEGRRDS
                                                        •
601  LFIPIRARER RSSYSGYSGY SQCSRSSRIF PSLRRSVKRN STVDCNGVVS
                  •                              •    ◆
651  LIGPGSHIGR LLPEATTEVE IKKKGPGSLL VSMDQLASYG RKDRINSIMS

701  VVTNTLVEEL EESQRKCPPC WYKFANTFLI WECHPYWIKL KEIVNLIVMD
                                                  |------
751  PFVDLAITIC IVLNTLFMAM EHHPMTPQFE HVLAVGNLVF TGIFTAEMFL
     ----IIS1------------|           |----------IIS2-----
801  KLIAMDPYYY FQEGWNIFDG FIVSLSLMEL SLADVEGLSV LRSFRLLRVF
     ----|          |----------IIS3-------|      |--------IIS4
```

Fig. 1B: SEQ ID NO:3

```
 851  KLAKSWPTLN MLIKIIGNSV GALGNLTLVL AIIVFIFAVV GMQLFGKSYK
      --------|                  ◆ |---------IIS5---------|
 901  ECVCKINQEC KLPRWHMNDF FHSFLIVFRV LCGEWIETMW DCMEVAGQAM
                                                           |-
 951  CLIVFMMVMV IGNLVVLNLF LALLLSSFSA DNLAATDDDG EMNNLQISVI
      -----------IIS6-----------|
1001  RIKKGVAWTK VKVHAFMQAH FKQREADEVK PLDELYEKKA NCIANHTGVD
                                                        ◆
1051  IHRNGDFQKN GNGTTSGIGS SVEKYIIDED HMSFINNPNL TVRVPIAVGE
                   ◆                            ◆
1101  SDFENLNTED VSSESDPEGS KDKLDDTSSS EGSTIDIKPE VEEVPVEQPE

1151  EYLDPDACFT EGCVQRFKCC QVNIEEGLGK SWWILRKTCF LIVEHNWFET
                                                     |-------
1201  FIIFMILLSS GALAFEDIYI EQRKTIRTIL EYADKVFTYI FILEMLLKWT
      ---IIIS1-------|              |----------IIIS2---------
1251  AYGFVKFFTN AWCWLDFLIV AVSLVSLIAN ALGYSELGAI KSLRTLRALR
      --|         |----------IIIS3---------|   |--------IIIS4-
1301  PLRALSRFEG MRVVVNALVG AIPSIMNVLL VCLIFWLIFS IMGVNLFAGK
      --------|                     |----------IIIS5--------|
1351  YHYCFNETSE IRFEIDIVNN KTDCEKLMEG NSTEIRWKNV KINFDNVGAG
            ◆              ◆            ◆
1401  YLALLQVATF KGWMDIMYAA VDSRKPDEQP DYEGNIYMYI YFVIFIIFGS
                                             |-----------IIIS6
1451  FFTLNLFIGV IIDNFNQQKK KFGGQDIFMT EEQKKYYNAM KKLGSKKPQK
      ------------|
1501  PIPRPLNKIQ GIVFDFVTQQ AFDIVIMMLI CLNMVTMMVE TDTQSKQMEN
                       |----------IVS1----------|
1551  ILYWINLVFV IFFTCECVLK MFALRHYYFT IGWNIFDFVV VILSIVGMFL
      |----------IVS2----------|         |--------------IVS3--
1601  ADIIEKYFVS PTLFRVIRLA RIGRILRLIK GAKGIRTLLF ALMMSLPALF
      ----|           |----------IVS4---------|
1651  NIGLLLFLVM FIFSIFGMSN FAYVKHEAGI DDMFNFETFG NSMICLFQIT
        |----------IVS5----------|
```

Fig. 1C: SEQ ID NO:3

```
1701  TSAGWDGLLL PILNRPPDCS LDKEHPGSGF KGDCGNPSVG IFFFVSYIII
                                                |-----------
1751  SFLIVVNMYI AIILENFSVA TEESADPLSE DDFETFYEIW EKFDPDATQF
      IVS6----------| ♦
1801  IEYCKLADFA DALEHPLRVP KPNTIELIAM DLPMVSGDRI HCLDILFAFT

1851  KRVLGDSGEL DILRQQMEER FVASNPSKVS YEPITTTLRR KQEEVSAVVL

1901  QRAYRGHLAR RGFICRKMAS NKLENGGTHR DKKESTPSTA SLPSYDSVTK

1951  PDKEKQQRAE EGRRERAKRQ KEVRESKC
```

Fig. 2A:-SEQ ID NO:4

```
  1  MAARLLAPPG PDSFKPFTPE SLANIERRIA ESKLKKPPKA DGSHREDDED

 51  SKPKPNSDLE AGKSLPFIYG DIPQGLVAVP LEDFDPYYLT QKTFVVLNRG

101  KTLFRFSATP ALYILSPFNL IRRIAIKILI HSVFSMIIMC TILTNCVFMT
                               |----------IS1--------------|
151  FSNPPEWSKN VEYTFTGIYT FESLVKIIAR GFCIDGFTFL RDPWNWLDFS
              |----------IS2--------|              |-------
201  VIMMAYVTEF VDLGNVSALR TFRVLRALKT ISVIPGLKTI VGALIQSVKK
     IS3--------|    ◆   |-------IS4----------|
251  LSDVMILTVF CLSVFALIGL QLFMGNLRNK CVVWPINFNE SYLENGTRGF
      •|-----------IS5-----------|             ◆      ◆
301  DWEEYINNKT NFYMVPGMLE PLLCGNSSDA GQCPEGFQCM KAGRNPNYGY
             ◆                   ◆
351  TSFDTFSWAF LALFRLMTQD YWENLYQLTL RAAGKTYMIF FVLVIFVGSF
                            Δ                 |-----------IS6
401  YLVNLILAVV AMAYEEQNQA TLEEAEQKEA EFKAMLEQLK KQQEEAQAAA
     ------------|
451  MATSAGTVSE DAIEEEGEDG VGSPRSSSEL SKLSSKSAKE RRNRRKKRKQ

501  KELSEGEEKG DPEKVFKSES EDGMRRKAFR LPDNRIGRKF SIMNQSLLSI
                                                  •  ◆
551  PGSPFLSRHN SKSSIFSFRG PGRFRDPGSE NEFADDEHST VEESEGRRDS
                                                          •
601  LFIPIRARER RSSYSGYSGY SQCSRSSRIF PSLRRSVKRN STVDCNGVVS
                  •                             ◆ •
651  LIGPGSHIGR LLPEVKIDKA ATDSATTEVE IKKKGPGSLL VSMDQLASYG

701  RKDRINSIMS VVTNTLVEEL EESQRKCPPC WYKFANTFLI WECHPYWIKL

751  KEIVNLIVMD PFVDLAITIC IVLNTLFMAM EHHPMTPQFE HVLAVGNLVF
         |-----------IIS1------------|             |--------
801  TGIFTAEMFL KLIAMDPYYY FQEGWNIFDG FIVSLSLMEL SLADVEGLSV
     IIS2-----------|         |----------IIS3-------|     |
```

Fig. 2B: SEQ ID NO:4

```
851   LRSFRLLRVF KLAKSWPTLN MLIKIIGNSV GALGNLTLVL AIIVFIFAVV
      ---------IIS4------|                      ♦|----------IIS5-
901   GMQLFGKSYK ECVCKINQEC KLPRWHMNDF FHSFLIVFRV LCGEWIETMW
      -------|
951   DCMEVAGQAM CLIVFMMVMV IGNLVVLNLF LALLLSSFSA DNLAATDDDG
              |------------IIS6-------------|
1001  EMNNLQISVI RIKKGVAWTK VKVHAFMQAH FKQREADEVK PLDELYEKKA

1051  NCIANHTGVD IHRNGDFQKN GNGTTSGIGS SVEKYIIDED HMSFINNPNL
          ♦                   ♦                           ♦
1101  TVRVPIAVGE SDFENLNTED VSSESDPEGS KDKLDDTSSS EGSTIDIKPE

1151  VEEVPVEQPE EYLDPDACFT EGCVQRFKCC QVNIEEGLGK SWWILRKTCF

1201  LIVEHNWFET FIIFMILLSS GALAFEDIYI EQRKTIRTIL EYADKVFTYI
        |-----------IIIS1--------|            |------------
1251  FILEMLLKWT AYGFVKFFTN AWCWLDFLIV AVSLVSLIAN ALGYSELGAI
      IIIS2---------|         |-----------IIIS3--------|   |--
1301  KSLRTLRALR PLRALSRFEG MRVVVNALVG AIPSIMNVLL VCLIFWLIFS
      --------IIIS4------|                    |---------IIIS5
1351  IMGVNLFAGK YHYCFNETSE IRFEIDIVNN KTDCEKLMEG NSTEIRWKNV
      --------|       ♦               ♦               ♦
1401  KINFDNVGAG YLALLQVATF KGWMDIMYAA VDSRKPDEQP DYEGNIYMYI
                                                       |---
1451  YFVIFIIFGS FFTLNLFIGV IIDNFNQQKK KFGGQDIFMT EEQKKYYNAM
      --------IIIS6----------|
1501  KKLGSKKPQK PIPRPLNKIQ GIVFDFVTQQ AFDIVIMMLI CLNMVTMMVE
                              |---------IVS1-----------|
1551  TDTQSKQMEN ILYWINLVFV IFFTCECVLK MFALRHYYFT IGWNIFDFVV
                 |-----------IVS2----------|    |---------
1601  VILSIVGMFL ADIIEKYFVS PTLFRVIRLA RIGRILRLIK GAKGIRTLLF
      ---IVS3--------|        |---------IVS4----------|
1651  ALMMSLPALF NIGLLLFLVM FIFSIFGMSN FAYVKHEAGI DDMFNFETFG
                 |--------IVS5------------|
```

Fig. 2C: SEQ ID NO:4

```
1701  NSMICLFQIT TSAGWDGLLL PILNRPPDCS LDKEHPGSGF KGDCGNPSVG
                                                        |-
1751  IFFFVSYIII SFLIVVNMYI AIILENFSVA TEESADPLSE DDFETFYEIW
      ------------IVS6----------|◆
1801  EKFDPDATQF IEYCKLADFA DALEHPLRVP KPNTIELIAM DLPMVSGDRI

1851  HCLDILFAFT KRVLGDSGEL DILRQQMEER FVASNPSKVS YEPITTTLRR

1901  KQEEVSAVVL QRAYRGHLAR RGFICRKMAS NKLENGGTHR DKKESTPSTA
                                                   •
1951  SLPSYDSVTK PDKEKQQRAE EGRRERAKRQ KEVRESKC
```

Fig. 3A: NaCh6/PN4 alignment (SEQ ID NO:7)

```
RATNaCh6A   ---------- ---------- ---------- ---------- ----------
rPN4        CCAAGATGGC GCCCACCGCA GTCCCGCCCG CCGCAGCCTC GGCGCCTCTG    50

RATNaCh6A   ---------- ---------- ---------- ---------- ----------
rPN4        CAGTCCGGCC GCGCCTCCCG GGCCCCGCGC TAGGGCCGCT GCCGCCTCGC   100

RATNaCh6A   ---------- ---------- ---------- ---------- ----------
rPN4        CCGCCGCCGC CGCCGCCAGC TGACCTGTCC CGGACACATA ACTAACGAAG   150

                       start⇒
RATNaCh6A   ---------- ATGAGAAGAT --CGGCGCGG CTGCTCGCAC CACCAGGCCC    38
rPN4        CTGCTGCAGG ATGAGAAGAT GGCAGCGCGG CTGCTCGCAC CACCAGGCCC   200
                                start⇒

RATNaCh6A   TGATAGTTTC AAGCCTTTCA CCCCTGAGTC GCTGGCAAAC ATCGAGAGGC    88
rPN4        TGATAGTTTC AAGCCTTTCA CCCCTGAGTC GCTGGCAAAC ATCGAGAGGC   250

RATNaCh6A   GTATTGCCGA GAGCAAGCTC AAGAAACCAC CAAAGGCGGA TGGCAGCCAC   138
rPN4        GTATTGCCGA GAGCAAGCTC AAGAAACCAC CAAAGGCGGA TGGCAGCCAC   300

RATNaCh6A   CGGGAGGACG ATGAAGACAG CAAGCCCAAG CCAAACAGTG ACCTGGAGGC   188
rPN4        CGGGAGGACG ATGAAGACAG CAAGCCCAAG CCAAACAGTG ACCTGGAGGC   350

RATNaCh6A   TGGGAAGAGT TTGCCTTTCA TCTACGGGGA CATCCCGCAA GGCCTGGTTG   238
rPN4        TGGGAAGAGT TTGCCTTTCA TCTACGGGGA CATCCCGCAA GGCCTGGTTG   400

RATNaCh6A   CGGTTCCCCT GGAGGACTTT GACCCTTACT ATTTGACGCA GAAAACCTTT   288
rPN4        CGGTTCCCCT GGAGGACTTT GACCCTTACT ATTTGACGCA GAAAACCTTT   450

RATNaCh6A   GTAGTATTAA ACAGAGGGAA AACTCTCTTC AGATTTAGTG CCACACCTGC   338
rPN4        GTAGTATTAA ACAGAGGGAA AACTCTCTTC AGATTTAGTG CCACACCTGC   500

RATNaCh6A   CTTGTACATT TTAAGCCCTT TTAACCTGAT AAGAAGAATA GCTATTAAAA   388
rPN4        CTTGTACATT TTAAGCCCTT TTAACCTGAT AAGAAGAATA GCTATTAAAA   550

RATNaCh6A   TTTTGATACA CTCAGTTTTC AGCATGATCA TCATGTGCAC CATCCTGACC   438
rPN4        TTTTGATACA CTCAGTTTTC AGCATGATCA TCATGTGCAC CATCCTGACC   600

RATNaCh6A   AACTGTGTGT TCATGACCTT TAGTAACCCT CCAGAATGGT CCAAGAATGT   488
rPN4        AACTGTGTGT TCATGACCTT TAGTAACCCT CCAGAATGGT CCAAGAATGT   650

RATNaCh6A   GGAGTACACA TTCACAGGGA TTTACACATT TGAATCACTA GTGAAAATCA   538
rPN4        GGAGTACACA TTCACAGGGA TTTACACATT TGAATCACTA GTGAAAATCA   700

RATNaCh6A   TCGCAAGAGG TTTCTGCATA GACGGCTTCA CCTTCTTACG AGACCCGTGG   588
rPN4        TCGCAAGAGG TTTCTGCATA GACGGCTTCA CCTTCTTGCG AGACCCGTGG   750

RATNaCh6A   AACTGGTTAG ACTTCAGTGT CATCATGATG GCATATGTGA CAGAGTTTGT   638
rPN4        AACTGGTTAG ACTTCAGTGT CATCATGATG GCATATGTGA CAGAGTTTGT   800

RATNaCh6A   GGACCTGGGC AATGTCTCAG CGCTGAGAAC ATTCAGGGTT CTCCGAGCTT   688
rPN4        GGACCTGGGC AATGTCTCAG CGCTGAGAAC ATTCAGGGTT CTCCGAGCTT   850
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Fig. 3B: NaCh6/PN4 alignment (SEQ ID NO:7)

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RATNaCh6A   TGAAAACTAT CTCTGTAATT CCAGGCCTGA AGACAATCGT GGGCGCCCTA    738
rPN4        TGAAAACTAT CTCTGTAATT CCAGGCCTGA AGACAATCGT GGGCGCCCTA    900

RATNaCh6A   ATCCAGTCCG TGAAGAAGCT GTCGGACGTG ATGATCCTGA CAGTGTTCTG    788
rPN4        ATCCAGTCCG TGAAGAAGCT GTCGGACGTG ATGATCCTGA CAGTGTTCTG    950

RATNaCh6A   CCTGAGTGTT TTCGCCCTGA TTGGCCTGCA GCTCTTTCAT GGGAACCTTT    838
rPN4        CCTGAGTGTT TTCGCCCTGA TTGGCCTGCA GCTCTTCATG GGGAACCTT-    999

RATNaCh6A   CGAAAC-AGT GTGTCGTGTG GCCCATAAAC TTCAACGAGA GCTACCTGGA    887
rPN4        CGAAACAAGT GTGTCGTGTG GCCCATAAAC TTCAACGAGA GCTACCTGGA    1049

RATNaCh6A   GAACGGCACC AGAGGCTTTG ACTGGGAGGA ATATATCAAC AATAAAACAA    937
rPN4        GAACGGCACC AGAGGCTTTG ACTGGGAGGA ATATATCAAC AATAAAACAA    1099

RATNaCh6A   ACTTTTACAT GGTTCCTGGC ATGCTAGAAC CCTTGCTCTG CGGGAACAGT    987
rPN4        ACTTTTACAT GGTTCCTGGC ATGCTAGAAC CCTTGCTCTG CGGGAACAGT    1149

RATNaCh6A   TCTGATGCTG GGCAATGC-- -GAAGGATTC CAGTGCAGTA AAGCAGGAAG    1034
rPN4        TCTGATGCTG GGCAATGCCC AGAGGGATTC CAGTGCATGA AAGCAGGAAG    1199

RATNaCh6A   GAACCCCAAC TACGGTTACA CCAGCTTTGA CACCTTCAGC TGGGCCTTCT    1084
rPN4        GAACCCCAAC TACGGTTACA CCAGCTTTGA CACCTTCAGC TGGGCCTTCT    1249

RATNaCh6A   TGGCATTATT CCGCCTTATG ACCCAGGACT ATTGGGAGAA CTTATACCAG    1134
rPN4        TGGCATTATT CCGCCTTATG ACCCAGGACT ATTGGGAGAA CTTATACCAG    1299

RATNaCh6A   CTGACCTTAC GAGCCGCTGG GAAAACGTAC ATGATCTTCT TTGTCTTGGT    1184
rPN4        CTGACCTTAC GAGCCGCTGG GAAAACGTAC ATGATCTTCT TTGTCTTGGT    1349

RATNaCh6A   CATCTTCGTG GGTTCTTTCT ATCCGGTGAA CTTGATCTTG GCTGTGGTGG    1234
rPN4        CATCTTCGTG GGTTCTTTCT ATCTGGTGAA CTTGATCTTG GCTGTGGTGG    1399

RATNaCh6A   CCATGGCTTA TGAGGAACAG AACCAGGCAA CACTGGAGGA GGCAGAGCAA    1284
rPN4        CCATGGCTTA TGAGGAACAG AACCAGGCAA CACTGGAGGA GGCAGAGCAA    1449

RATNaCh6A   AAAGAGGCCG AGTTCAAGGC AATGCTGGAG CAACTCAAGA AGCAGCAGGA    1334
rPN4        AAAGAGGCCG AGTTCAAGGC AATGCTGGAG CAACTCAAGA AGCAGCAGGA    1499

RATNaCh6A   GGAGGCACAG GCTGCTGCAA TGGCCACCTC AGCGGGCACT GTCTCGGAAG    1384
rPN4        GGAGGCACAG GCTGCTGCAA TGGCCACCTC AGCGGGCACT GTCTCGGAAG    1549

RATNaCh6A   ACGCCATTGA AGAAGAAGGG GAAGATGGGG TAGGCTCTCC GAGGAGCTCT    1434
rPN4        ACGCCATTGA AGAAGAAGGG GAAGATGGGG TAGGCTCTCC GAGGAGCTCT    1599

RATNaCh6A   TCTGAACTGT CTAAACTCAG TTCCAAGAGC GCGAAGGAGC GGCGGAACCG    1484
rPN4        TCTGAACTGT CTAAACTCAG TTCCAAGAGC GCGAAGGAGC GGCGGAACCG    1649

RATNaCh6A   ACGGAAGAAG AGGAAGCAGA AGGAGCTCTC TGAAGGCGAG GAGAAAGGGG    1534
rPN4        ACGGAAGAAG AGGAAGCAGA AGGAGCTCTC TGAAGGCGAG GAGAAAGGGG    1699
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Fig. 3C: NaCh6/PN4 alignment (SEQ ID NO:7)

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RATNaCh6A   ACCCGGAGAA GGTGTTTAAG TCAGAGTCGG AATACGGTAT GAGAAGGAAG   1584
rPN4        ACCCGGAGAA GGTGTTTAAG TCAGAGTCGG AAGACGGTAT GAGAAGGAAG   1749

RATNaCh6A   GCCTTCCGGC TGCCAGACAA CAGGATAGGG AGGAAGTTTT CCATCATGAA   1634
rPN4        GCCTTCCGGC TGCCAGACAA CAGGATAGGG AGGAAGTTTT CCATCATGAA   1799

RATNaCh6A   TCAGTCGCTG CTCAGCATTC CAGGCTCGCC CTTCCTCTCC CGACATAACA   1684
rPN4        TCAGTCGCTG CTCAGCATTC CAGGCTCGCC CTTCCTCTCC CGACATAACA   1849

RATNaCh6A   GCAAAAGCAG CATCTTCAGC TTC-GGGGAC CC-GTCGGTT -CGGGACCCC   1731
rPN4        GCAAAAGCAG CATCTTCAGC TTCCGGGGAC CCGGTCGGTT CCGGGACCCC   1899

RATNaCh6A   GGCTCTGAGA ATGAGTTCGC AGACGATGAA CACAGCACCG TGGAGGAGAG   1781
rPN4        GGCTCTGAGA ATGAGTTCGC AGACGATGAA CACAGCACCG TGGAGGAGAG   1949

RATNaCh6A   CGAGGGCCGG CGTGACTCGC TCTTCATCCC GATCCGCGCC CGCGAGCGCC   1831
rPN4        CGAGGGCCGG CGTGACTCGC TCTTCATCCC GATCCGCGCC CGCGAGCGCC   1999

RATNaCh6A   GCAGCAGCTA CAGTGGCTAC AGCGGCTACA GCCAGTGCAG CCGCTCGTCG   1881
rPN4        GCAGCAGCTA CAGTGGCTAC AGCGGCTACA GCCAGTGCAG CCGCTCGTCG   2049

RATNaCh6A   CGCATCT-CC CCAGCCTGC- GCGCAGCGTG AAGC-CAACA GCACGGTGGA   1928
rPN4        CGCATCTTCC CCAGCCTGCG GCGCAGCGTG AAGCGCAACA GCACGGTGGA   2099

RATNaCh6A   CTGCAACGGC GTAGTGTCAC TCATCGGGCC CGGCTCACAC ATCGGGCGGC   1978
rPN4        CTGCAACGGC GTAGTGTCAC TCATCGGGCC CGGCTCACAC ATCGGGCGGC   2149

RATNaCh6A   TCCTGC-TGA GGCAACGACT GAGGTGGAAA TTAAGAAGAA AGGCCCTGGA   2027
rPN4        TCCTGCCTGA GGCAACGACT GAGGTGGAAA TTAAGAAGAA AGGCCCTGGA   2199

RATNaCh6A   -CTCTTTTAG TTTCTATGGA CCAACTCGCC TCCTACGGAC GGAAGGACAG   2076
rPN4        TCTCTTTTAG TTTCTATGGA CCAACTCGCC TCCTACGGAC GGAAGGACAG   2249

RATNaCh6A   AATCAACAGC ATAATGAGCG TGGTCACAAA CACGCTAGT- GAAGAGCTGG   2125
rPN4        AATCAACAGC ATAATGAGCG TGGTCACAAA CACGCTAGTG GAAGAGCTGG   2299

RATNaCh6A   AAGAGTCTCA GAGAAAGTGC CCACCGTGCT GGTATAAGTT TGCCAACACT   2175
rPN4        AAGAGTCTCA GAGAAAGTGC CCACCGTGCT GGTATAAGTT TGCCAACACT   2349

RATNaCh6A   TTCCTCATCT GGGAGTGTCA CCCCTACTGG ATAAAACTGA AGGAGATCGT   2225
rPN4        TTCCTCATCT GGGAGTGTCA CCCCTACTGG ATAAAACTGA AGGAGATCGT   2399

RATNaCh6A   GAACTTAATC GTCATGGACC CTTTTGTAGA CTTAGCCATC ACCATCTGCA   2275
rPN4        GAACTTAATC GTCATGGACC CTTTTGTAGA CTTAGCCATC ACCATCTGCA   2449

RATNaCh6A   TCGTTCTGAA TACGCTATTT ATGGCAATGG AGCACCATCC CATGACACCA   2325
rPN4        TCGTTCTGAA TACGCTATTT ATGGCAATGG AGCACCATCC CATGACACCA   2499

RATNaCh6A   CAGTTCGAAC ACGTCTTGGC CGTAGGAAAT CTGGTGTTCA CCGGGATCTT   2375
rPN4        CAGTTCGAAC ACGTCTTGGC CGTAGGAAAT CTGGTGTTCA CCGGGATCTT   2549

RATNaCh6A   CACGGCGGAA ATGTTTCTGA AGCTCATAGC CATGGACCCC TACTATTATT   2425
rPN4        CACGGCGGAA ATGTTTCTGA AGCTCATAGC CATGGACCCC TACTATTATT   2599
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Fig. 3D: NaCh6/PN4 alignment (SEQ ID NO:7)

RATNaCh6A	TCCAAGAAGG CTGGAACATT TTTGACGGAT TTATTGTCTC CCTCAGTTTA	2475
rPN4	TCCAAGAAGG CTGGAACATT TTTGACGGAT TTATTGTCTC CCTCAGTTTA	2649
RATNaCh6A	ATGGAGCTGA GTCTCGCAGA TGTGGAGGGG CTCTCAGTGC TGCGGTCTTT	2525
rPN4	ATGGAGCTGA GTCTCGCAGA TGTGGAGGGG CTCTCAGTGC TGCGGTCTTT	2699
RATNaCh6A	CCGACTGCTC CGAGTCTTCA AGCTGGCCAA GTCCTGGCCC ACCCTGAACA	2575
rPN4	CCGACTGCTC CGAGTCTTCA AGCTGGCCAA GTCCTGGCCC ACCCTGAACA	2749
RATNaCh6A	TGCTGATCAA GATCATCGGG AACTCCGTGG GTGCCCTGGG CAACCTGACC	2625
rPN4	TGCTGATCAA GATCATCGGG AACTCCGTGG GTGCCCTGGG CAACCTGACC	2799
RATNaCh6A	CTGGTGCTGG CCATCATCGT CTTCATCTTC GCCGTGGTGG GGATGCAGCT	2675
rPN4	CTGGTGCTGG CCATCATCGT CTTCATCTTC GCCGTGGTGG GGATGCAGCT	2849
RATNaCh6A	GTTTGGAAAG AGTTACAAGG AGTGCGTCTG TAAGATCAAC CAGGAGTGCA	2725
rPN4	GTTTGGAAAG AGTTACAAGG AGTGCGTCTG TAAGATCAAC CAGGAGTGCA	2899
RATNaCh6A	AGCTCCCGCG CTGGCACATG AACGACTTCT TCCACTCCTT CCTCATCGTC	2775
rPN4	AGCTCCCGCG CTGGCACATG AACGACTTCT TCCACTCCTT CCTCATCGTC	2949
RATNaCh6A	TTCCGAGTGC TGTGTGGGGA GTGGATCGAG ACCATGTGGG ACTGCATGGA	2825
rPN4	TTCCGAGTGC TGTGTGGGGA GTGGATCGAG ACCATGTGGG ACTGCATGGA	2999
RATNaCh6A	GGTGGCCGGC CAGGCCATGT GCCTCATTGT CTTCATGATG GTTATGGTCA	2875
rPN4	GGTGGCCGGC CAGGCCATGT GCCTCATTGT CTTCATGATG GTTATGGTCA	3049
RATNaCh6A	TTGGCAACCT GGTGGTGCTG AATCTATTCC TGGCCTTGCT TCTGAGCTCC	2925
rPN4	TTGGCAACCT GGTGGTGCTG AATCTATTCC TGGCCTTGCT TCTGAGCTCC	3099
RATNaCh6A	TTCAGCGCAG ACAACCTGGC GGCCACAGAC GACGACGGGG AAATGAACAA	2975
rPN4	TTCAGCGCAG ACAACCTGGC GGCCACAGAC GACGACGGGG AAATGAACAA	3149
RATNaCh6A	CCTGCAGATC TCAGTGATCC GGATCAAGAA GGGCGTGGCC TGGACCAAAG	3025
rPN4	CCTGCAGATC TCAGTGATCC GGATCAAGAA GGGCGTGGCC TGGACCAAAG	3199
RATNaCh6A	TGAAGGTGCA CGCCTTCATG CAGGCTCACT TCAAGCAGCG GGAGGCGGAT	3075
rPN4	TGAAGGTGCA CGCCTTCATG CAGGCTCACT TCAAGCAGCG GGAGGCGGAT	3249
RATNaCh6A	GAAGTGAAAC CCCTCGACGA GCTGTATGAG AAGAAGGCCA ACTGCATCGC	3125
rPN4	GAAGTGAAAC CCCTCGACGA GCTGTATGAG AAGAAGGCCA ACTGCATCGC	3299
RATNaCh6A	CAACCACACG GGCGTGGATA TCCACCGGAA CGGCGACTTC CAGAAGAACG	3175
rPN4	CAACCACACG GGCGTGGATA TCCACCGGAA CGGCGACTTC CAGAAGAACG	3349
RATNaCh6A	GGAACGGAAC CACCAGCGGC ATCGGCAGCA GCGTGGAGAA GTACATCATC	3225
rPN4	GGAACGGAAC CACCAGCGGC ATCGGCAGCA GCGTGGAGAA GTACATCATC	3399
RATNaCh6A	GACGAGGACC ACATGTCCTT CATTAACAAC CCAAACCTGA CCGTCCGGGT	3275
rPN4	GACGAGGACC ACATGTCCTT CATTAACAAC CCAAACCTGA CCGTCCGGGT	3449
RATNaCh6A	GCCCATTGCT GTGGGCGAGT CTGACTTCGA GAACCTCAAC ACAGAGGATG	3325
rPN4	GCCCATTGCT GTGGGCGAGT CTGACTTCGA GAACCTCAAC ACAGAGGATG	3499

Fig. 3E: NaCh6/PN4 alignment (SEQ ID NO:7)

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RATNaCh6A   TTAGCAGCGA ATCAGACCCT GAAGGCAGCA AAGATAAACT GGACGATACC   3375
rPN4        TTAGCAGCGA ATCAGACCCT GAAGGCAGCA AAGATAAACT GGACGATACC   3549

RATNaCh6A   AGCTCCTCAG AAGGAAGTAC CATCGACATC AAGCCTGAGG TGGAAGAAGT   3425
rPN4        AGCTCCTCAG AAGGAAGTAC CATCGACATC AAGCCTGAGG TGGAAGAAGT   3599

RATNaCh6A   TCCCGTGGAG CAACCTGAGG AATACTTGGA TCCGGACGCC TGCTTTACAG   3475
rPN4        TCCCGTGGAG CAACCTGAGG AATACTTGGA TCCGGACGCC TGCTTTACAG   3649

RATNaCh6A   AGGGTTGCGT CCAGCGGTTC AAGTGCTGCC AGGTCAACAT CGAGGAAGGA   3525
rPN4        AGGGTTGCGT CCAGCGGTTC AAGTGCTGCC AGGTCAACAT CGAGGAAGGA   3699

RATNaCh6A   CTAGGCAAGT CGTGGTGGAT CTTGCGGAAA ACCTGCTTCC TCATTGTGGA   3575
rPN4        CTAGGCAAGT CGTGGTGGAT CTTGCGGAAA ACCTGCTTCC TCATTGTGGA   3749

RATNaCh6A   GCACAATTGG TTTGAGACCT TCATCATCTT CATGATTCTG CTCAGCAGTG   3625
rPN4        GCACAATTGG TTTGAGACCT TCATCATCTT CATGATTCTG CTCAGCAGTG   3799

RATNaCh6A   GCGCCCTGGC CTTTGAGGAC ATCTACATTG AGCAGAGGAA GACCATCCGC   3675
rPN4        GCGCCCTGGC CTTTGAGGAC ATCTACATTG AGCAGAGGAA GACCATCCGC   3849

RATNaCh6A   ACCATCCTGG AGTATGCGGA CAAGGTCTTC ACCTACATCT TCATCCTGGA   3725
rPN4        ACCATCCTGG AGTATGCGGA CAAGGTCTTC ACCTACATCT TCATCCTGGA   3899

RATNaCh6A   GATGTTGCTC AAGTGGACCA CGTACGGCTT CGTCAAGTTC TTCACCAATG   3775
rPN4        GATGTTGCTC AAGTGGACAG CCTACGGCTT CGTCAAGTTC TTCACCAATG   3949

RATNaCh6A   CCTGGTGCTG GTTGGACTTC CTCATTGTGG CTGTCTCTTT AGTCAGCCTT   3825
rPN4        CCTGGTGCTG GTTGGACTTC CTCATTGTGG CTGTCTCTTT AGTCAGCCTT   3999

RATNaCh6A   ATAGCTAATG CCCTGGGCTA CTCGGAACTA GGTGCCATAA AGTCCCTTAG   3875
rPN4        ATAGCTAATG CCCTGGGCTA CTCGGAACTA GGTGCCATAA AGTCCCTTAG   4049

RATNaCh6A   GACCCTAAGA GCTTTGAGAC CCTTAAGAGC CTTATCACGA TTTGAAGGGA   3925
rPN4        GACCCTAAGA GCTTTGAGAC CCTTAAGAGC CTTATCACGA TTTGAAGGGA   4099

RATNaCh6A   TGAGGGTGGT GGTGAATGCC TTGGTGGGTG CCATCCCCTC CATCATGAAT   3975
rPN4        TGAGGGTGGT GGTGAATGCC TTGGTGGGCG CCATCCCCTC CATCATGAAT   4149

RATNaCh6A   GTGCTGCTGG TGTGTCTCAT CTTCTGGCTG ATTTTCAGCA TCATGGGAGT   4025
rPN4        GTGCTGCTGG TGTGTCTCAT CTTCTGGCTG ATTTTCAGCA TCATGGGAGT   4199

RATNaCh6A   TAACCTGTTT GCGGGGAAAT ACCACTACTG CTTTAATGAG ACTTCTGAAA   4075
rPN4        TAACCTGTTT GCGGGGAAAT ACCACTACTG CTTTAATGAG ACTTCTGAAA   4249

RATNaCh6A   TCCGGTTCGA AATCGATATT GTCAACAATA AAACGGACTG TGAGAAGCTC   4125
rPN4        TCCGGTTCGA AATCGATATT GTCAACAATA AAACGGACTG TGAGAAGCTC   4299

RATNaCh6A   ATGGAGGGCA ACAGCACGGA GATCCGATGG AAGAATGTCA AGATCAACTT   4175
rPN4        ATGGAGGGCA ACAGCACGGA GATCCGATGG AAGAATGTCA AGATCAACTT   4349

RATNaCh6A   TGACAATGTC GGAGCAGGGT ACCTGGCCCT TCTTCAAGTG GCAACCTTCA   4225
rPN4        TGACAATGTC GGAGCAGGGT ACCTGGCCCT TCTTCAAGTG GCAACCTTCA   4399
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Fig. 3F: NaCh6/PN4 alignment (SEQ ID NO:7)

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RATNaCh6A   AAGGCTGGAT GGACATCATG TATGCGGCTG TAGATTCCCG AAAGCCAGAC   4275
rPN4        AAGGCTGGAT GGACATCATG TATGCGGCTG TAGATTCCCG AAAGCCAGAC   4449

RATNaCh6A   GAGCAGCCTG ACTACGAGGG CAACATCTAC ATGTACATCT ACTTCGTCAT   4325
rPN4        GAGCAGCCTG ACTACGAGGG CAACATCTAC ATGTACATCT ACTTCGTCAT   4499

RATNaCh6A   CTTCATCATC TTCGGCTCCT TCTTCACCCT CAACCTGTTC ATCGGTGTCA   4375
rPN4        CTTCATCATC TTCGGCTCCT TCTTCACCCT CAACCTGTTC ATCGGTGTCA   4549

RATNaCh6A   TCATCGACAA CTTCAACCAG CAGAAGAAAA AGTTTGGAGG TCAGGACATC   4425
rPN4        TCATCGACAA CTTCAACCAG CAGAAGAAAA AGTTTGGAGG TCAGGACATC   4599

RATNaCh6A   TTCATGACAG AGGAACAGAA GAAGTACTAT AATGCCATGA AAAAGCTGGG   4475
rPN4        TTCATGACAG AGGAACAGAA GAAGTACTAC AATGCCATGA AAAAGCTGGG   4649

RATNaCh6A   CTCCAAGAAG CCACAGAAGC CCATCCCCCG ACCCTTGAAC AAAATCCAAG   4525
rPN4        CTCCAAGAAG CCACAGAAGC CCATCCCCCG ACCCTTGAAC AAAATCCAAG   4699

RATNaCh6A   GGATTGTCTT TGATTTCGTC ACTCAACAAG CCTTTGACAT TGTGATCATG   4575
rPN4        GGATTGTCTT TGATTTCGTC ACTCAACAAG CCTTTGACAT TGTGATCATG   4749

RATNaCh6A   ATGCTCATCT GCCTTAACAT GGTGACAATG ATGGTGGAGA CAGACACTCA   4625
rPN4        ATGCTCATCT GCCTTAACAT GGTGACAATG ATGGTGGAGA CAGACACTCA   4799

RATNaCh6A   GAGCAAGCAG ATGGAGAACA TTCTTTACTG GATTAATCTG GTCTTTGTCA   4675
rPN4        GAGCAAGCAG ATGGAGAACA TTCTTTACTG GATTAATCTG GTCTTTGTCA   4849

RATNaCh6A   TCTTCTTCAC CTGCGAGTGT GTGCTCAAAA TGTTTGCCTT GAGACACTAC   4725
rPN4        TCTTCTTCAC CTGCGAGTGT GTGCTCAAAA TGTTTGCCTT GAGACACTAC   4899

RATNaCh6A   TACTTCACCA TTGGCTGGAA CATCTTTGAC TTTGTGGTGG TCATCCTCTC   4775
rPN4        TATTTCACCA TTGGCTGGAA CATCTTTGAC TTTGTGGTGG TCATCCTCTC   4949

RATNaCh6A   CATTGTGGGA ATGTTCCTGG CTGATATCAT TGAGAAGTAC TTCGTCTCCC   4825
rPN4        CATTGTGGGA ATGTTCCTGG CTGATATCAT TGAGAAGTAC TTCGTCTCCC   4999

RATNaCh6A   CAACCCTATT CCGAGTTATC CGATTGGCCC GTATTGGGCG CATCTTGCGT   4875
rPN4        CAACCCTATT CCGAGTTATC CGATTGGCCC GTATTGGGCG CATCTTGCGT   5049

RATNaCh6A   CTGATCAAGG GCGCCAAAGG GATCCGCACT CTGCTCTTTG CTCTGATGAT   4925
rPN4        CTGATCAAGG GCGCCAAAGG GATCCGCACC CTGCTCTTTG CCTTAATGAT   5099

RATNaCh6A   GTCGCTGCCC GCCCTGTTCA ACATCGGCCT CCTGCTCTTC CTCGTCATGT   4975
rPN4        GTCGCTGCCC GCCCTGTTCA ACATCGGCCT CCTGCTCTTC CTCGTCATGT   5149

RATNaCh6A   TCATCTTCTC CATTTTTGGC ATGTCCAACT TCGCATACGT GAAGCACGAG   5025
rPN4        TCATCTTCTC CATTTTTGGC ATGTCCAACT TCGCATACGT GAAGCACGAG   5199

RATNaCh6A   GCCGGCATTG ACGACATGTT CAACTTCGAG ACATTTGGCA ACAGCATGAT   5075
rPN4        GCCGGCATTG ACGACATGTT CAACTTCGAG ACATTTGGCA ACAGCATGAT   5249

RATNaCh6A   CTGTTTGTTC CAGATCACAA CGTCTGCTGG CTGGGATGGC CTGCTGCTGC   5125
rPN4        CTGTTTGTTC CAGATCACAA CGTCTGCTGG CTGGGATGGC CTGCTGCTGC   5299
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Fig. 3G: NaCh6/PN4 alignment (SEQ ID NO:7)

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RATNaCh6A   CAATCCTGAA CCGCCCCCCT GACTGCAGCT TGGACAAAGA GCACCCAGGG   5175
rPN4        CAATCCTGAA CCGCCCCCCT GACTGCAGCT TGGACAAAGA GCACCCAGGG   5349

RATNaCh6A   AGTGGCTTCA AAGGGGACTG TGGGAACCCC TCGGTGGGCA TCTTCTTCTT   5225
rPN4        AGTGGCTTCA AAGGGGACTG TGGGAACCCC TCGGTGGGCA TCTTCTTCTT   5399

RATNaCh6A   TGTGAGCTAC ATCATCATCT CCTTCCTGAT TGTGGTGAAC ATGTGCATCG   5275
rPN4        TGTGAGCTAC ATCATCATCT CCTTCCTGAT TGTGGTGAAC ATGTACATCG   5449

RATNaCh6A   CCATCATCCT GGAGAACTTC AGCGTGGCCA CCGAGGAGAG CGCCGACCCT   5325
rPN4        CCATCATCCT GGAGAACTTC AGCGTGGCCA CCGAGGAGAG CGCCGACCCT   5499

RATNaCh6A   CTGAGTGAGG ATGACTTCGA GACTTTCTAT GAGATCTGGG AGAAGTTTGA   5375
rPN4        CTGAGTGAGG ATGACTTCGA GACTTTCTAT GAGATCTGGG AGAAGTTTGA   5549

RATNaCh6A   CCCAGACGCC ACCCAGTTCA TCGAGTACTG TAAGCTGGCA GACTTTGCCG   5425
rPN4        CCCAGACGCC ACCCAGTTCA TCGAGTACTG TAAGCTGGCA GACTTTGCCG   5599

RATNaCh6A   ACGCCCTGGA GCACCCGCTC CGAGTACCCA AGCCCAACAC CATCGAGCTC   5475
rPN4        ACGCCCTGGA GCACCCGCTC CGAGTACCCA AGCCCAACAC CATCGAGCTC   5649

RATNaCh6A   ATCGCCATGG ACCTGCCCAT GGTGAGCGGA GATCGCATCC ACTGCTTGGA   5525
rPN4        ATCGCCATGG ACCTGCCCAT GGTGAGCGGA GATCGCATCC ACTGCTTGGA   5699

RATNaCh6A   CATCCTTTTC GCCTTCACCA AGGCAGTCCT GGGAGACAGT GGGGAGTTGG   5575
rPN4        CATCCTTTTC GCCTTCACCA AGCGAGTCCT GGGAGACAGT GGGGAGTTGG   5749

RATNaCh6A   ACATCCTGCG GCAGCAGATG GAGGAGCGGT TCGTGGCATC CAATCCTTCC   5625
rPN4        ACATCCTGCG GCAGCAGATG GAGGAGCGGT TCGTGGCATC CAATCCTTCC   5799

RATNaCh6A   AAAGTGTCTT ACGAAGCCTA TCAC-ACCAC TCTGCGGCGC AACGAGGAGG   5674
rPN4        AAAGTGTCTT ACGA-GCCTA TCACAACCAC TCTGCGGCGC AAGCAGGAGG   5848

RATNaCh6A   AGGTGTCTGC AGTGGTCCTG CAGCGTGCCT ACAGGGGACA CTTGGCTAGG   5724
rPN4        AGGTGTCTGC AGTGGTCCTG CAGCGTGCCT ACAGGGGACA CTTGGCTAGG   5898

RATNaCh6A   CGGGGCTTCA TCTGCAGAAA GATGGCCTCC AACAAGCTGG AGAATGGAGG   5774
rPN4        CGGGGCTTCA TCTGCAGAAA GATGGCCTCC AACAAGCTGG AGAATGGAGG   5948

RATNaCh6A   CACACACAGA GACAAGAAGG AGAGCACCCC GTCCACAGCC TCCCTCCCCT   5824
rPN4        CACACACAGA GACAAGAAGG AGAGCACCCC GTCCACAGCC TCCCTCCCCT   5998

RATNaCh6A   CTTACGACAG CGTCACAAAG CCAGACAAGG AGAAGCAGCA GCGTGCGGAG   5874
rPN4        CTTACGACAG CGTCACAAAG CCAGACAAGG AGAAGCAGCA GCGTGCGGAG   6048

RATNaCh6A   GAGGGCAGAA GGGAAAGAGC CAAGAGGCAA AAAGAGGTCA GGGAGTCCAA   5924
rPN4        GAGGGCAGAA GGGAAAGAGC CAAGAGGCAA AAAGAGGTCA GGGAGTCCAA   6098

                stop
RATNaCh6A   GTGCTAGAGG AGGGGAAAGG AAGCTTACCC CGGCTGAACA CTGGCAAGTG   5974
rPN4        GTGCTAGAGG AGGGGAAAGG AAGCTTACCC CGGCTGAACA CTGGCAAGTG   6148

RATNaCh6A   AAAGCTTGTT TACAAACTTC CGAATCTCAC GGATGCAGA- CAGCTGTGCA   6023
rPN4        AAAGCTTGTT TACAAACTTC CGAATCTCAC GGATGCAGAG CAGCTGTGCA   6198
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Fig. 3H: .NaCh6/PN4 alignment (SEQ ID NO:7)

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RATNaCh6A   GACGCTCGCT GTACTGGAAG ACCTATACCA AACATAGTCT GCTTACATGT   6073
rPN4        GACGCTCGCT GTACTGGAAG ACCTATACCA AACATAGTCT GCTTACATGT   6248

RATNaCh6A   GACATGGTGG CATCCTGAGC GGTGACTGCT GCTGGGGACA AAGGACCCTG   6123
rPN4        GACATGGTGG CATCCTGAGC GGTGA---CT GCTGGGGACA AAGGACCCTG   6295

RATNaCh6A   CTCCCTGGAC TCACAGATCT CCTATCGCTT GGGCAGACGG TTACTGCATG   6173
rPN4        CTCCCTGGAC TCACAGATCT CCTATCGCTT GGGCAGACGG TTACTGCATG   6345

RATNaCh6A   TTCCACACTT AGTCAATGCA ACTTAGGACT AAACTAACCA GGATACAAAA   6223
rPN4        TTCCACACTT AGTCAATGCA ACTTAGGACT AAACTAACCA GGATACAAAA   6395

RATNaCh6A   CCGAGGCGGC TG--GCGACC AGCAGATCAC CGCTGCAGCC AAATGGATTT   6271
rPN4        CCGAGGCGGC TGCCGGGACC AGCAGATCAC CGCTGCAGCC AAATGGATTT   6445

RATNaCh6A   TATTTTTTCA TTTTGTTGAT TCTCAGAAGC AGAAAGCATC ACTTTAAAAG   6321
rPN4        TATTTTTTCA TTTTGTTGAT TCTCAGAAGC AGAAAGCATC ACTTTAAAAG   6495

RATNaCh6A   TTTGTTTGTT CATGCAAACA ATATTTGAAT TCTTACATTA GTTAAGCTAA   6371
rPN4        TTTGTTTGTT CATNCAAACA ATATTTGAAT TCTTACATTA GTTAAGCTAA   6545

RATNaCh6A   GCAGCAAAAA GAAACACACA CGCACACAGA CACACAAAGA CACACACACA   6421
rPN4        GCANCAAAAA G--------- ---------- ---------- ----------   6556

RATNaCh6A   TTCAGCCTAT GTCACTAATC GTCTGTTTCT TTAACATAAC AGCATCTTCT   6471
rPN4        ---------- ---------- ---------- ---------- ----------   6556

RATNaCh6A   CCACACGAGC GGCACGTGGT TTGGAGATGG GTGGGGGAAA ATCAGGGTTT   6521
rPN4        ---------- ---------- ---------- ---------- ----------   6556

RATNaCh6A   CAGGCTGAGG AGGACTTGCT CAGGCCAATC CCAAATATGT GCTCGTTCAA   6571
rPN4        ---------- ---------- ---------- ---------- ----------   6556

RATNaCh6A   TGCATAGAAG TGACCTGCAT GATGGCATGC TGTGTTCAGA AGTCATGCAT   6621
rPN4        ---------- ---------- ---------- ---------- ----------   6556

RATNaCh6A   GAGACCCACA CACCACAAGA CACTAGTACT CCTGTNNCCA TCCACAGGCT   6671
rPN4        ---------- ---------- ---------- ---------- ----------   6556

RATNaCh6A   CAGCCTGCGG ACAGGACCAG CCCTGCACCG TTCACTGTAT TTGGAGAAAT   6721
rPN4        ---------- ---------- ---------- ---------- ----------   6556

RATNaCh6A   GGTAAGAGTT CCACACCGGC TGCAGTCCTC TCAGTGTAGG ATTCTTTCGT   6771
rPN4        ---------- ---------- ---------- ---------- ----------   6556

RATNaCh6A   ACACCTCTGG GTAGGGAGAC ATAATTAACC AATTGACCAC TACCAACAAA   6821
rPN4        ---------- ---------- ---------- ---------- ----------   6556

RATNaCh6A   ACAAT 6825
rPN4        ----- 6556
```

15/23

Fig. 4A:- PN4a/PN4/NaCh6 alignment

```
rPN4a           M--AARLLAP PGPDSFKPFT PESLANIERR IAESKLKKPP KADGSHREDD    48
rPN4            M--AARLLAP PGPDSFKPFT PESLANIERR IAESKLKKPP KADGSHREDD    48
RATNaCh6A       MRRSARLLAP PGPDSFKPFT PESLANIERR IAESKLKKPP KADGSHREDD    50

rPN4a           EDSKPKPNSD LEAGKSLPFI YGDIPQGLVA VPLEDFDPYY LTQKTFVVLN    98
rPN4            EDSKPKPNSD LEAGKSLPFI YGDIPQGLVA VPLEDFDPYY LTQKTFVVLN    98
RATNaCh6A       EDSKPKPNSD LEAGKSLPFI YGDIPQGLVA VPLEDFDPYY LTQKTFVVLN    100

                                          [-----------IS1------------
rPN4a           RGKTLFRFSA TPALYILSPF NLIRRIAIKI LIHSVFSMII MCTILTNCVF    148
rPN4            RGKTLFRFSA TPALYILSPF NLIRRIAIKI LIHSVFSMII MCTILTNCVF    148
RATNaCh6A       RGKTLFRFSA TPALYILSPF NLIRRIAIKI LIHSVFSMII MCTILTNCVF    150

                -]         [--------IS2--------]            [------
rPN4a           MTFSNPPEWS KNVEYTFTGI YTFESLVKII ARGFCIDGFT FLRDPWNWLD    198
rPN4            MTFSNPPEWS KNVEYTFTGI YTFESLVKII ARGFCIDGFT FLRDPWNWLD    198
RATNaCh6A       MTFSNPPEWS KNVEYTFTGI YTFESLVKII ARGFCIDGFT FLRDPWNWLD    200

                --IS3---]          [---------IS4-------]
rPN4a           FSVIMMAYVT EFVDLGNVSA LRTFRVLRAL KTISVIPGLK TIVGALIQSV    248
rPN4            FSVIMMAYVT EFVDLGNVSA LRTFRVLRAL KTISVIPGLK TIVGALIQSV    248
RATNaCh6A       FSVIMMAYVT EFVDLGNVSA LRTFRVLRAL KTISVIPGLK TIVGALIQSV    250

                     [----------IS5---------]
rPN4a           KKLSDVMILT VFCLSVFALI GLQLFMGNLR NKCVVWPINF NESYLENGTR    298
rPN4            KKLSDVMILT VFCLSVFALI GLQLFMGNLR NKCVVWPINF NESYLENGTR    298
RATNaCh6A       KKLSDVMILT VFCLSVFALI GLQLFHGNLS KQCVVWPINF NESYLENGTR    300

rPN4a           GFDWEEYINN KTNFYMVPGM LEPLLCGNSS DAGQCPEGFQ CMKAGRNPNY    348
rPN4            GFDWEEYINN KTNFYMVPGM LEPLLCGNSS DAGQCPEGFQ CMKAGRNPNY    348
RATNaCh6A       GFDWEEYINN KTNFYMVPGM LEPLLCGNSS DAGQC-EGFQ CSKAGRNPNY    349

                                                      [---------IS6
rPN4a           GYTSFDTFSW AFLALFRLMT QDYWENLYQL TLRAAGKTYM IFFVLVIFVG    398
rPN4            GYTSFDTFSW AFLALFRLMT QDYWENLYQL TLRAAGKTYM IFFVLVIFVG    398
RATNaCh6A       GYTSFDTFSW AFLALFRLMT QDYWENLYQL TLRAAGKTYM IFFVLVIFVG    399

                -------------]
rPN4a           SFYLVNLILA VVAMAYEEQN QATLEEAEQK EAEFKAMLEQ LKKQQEEAQA    448
rPN4            SFYLVNLILA VVAMAYEEQN QATLEEAEQK EAEFKAMLEQ LKKQQEEAQA    448
RATNaCh6A       SFYPVNLILA VVAMAYEEQN QATLEEAEQK EAEFKAMLEQ LKKQQEEAQA    449

rPN4a           AAMATSAGTV SEDAIEEEGE DGVGSPRSSS ELSKLSSKSA KERRNRRKKR    498
rPN4            AAMATSAGTV SEDAIEEEGE DGVGSPRSSS ELSKLSSKSA KERRNRRKKR    498
RATNaCh6A       AAMATSAGTV SEDAIEEEGE DGVGSPRSSS ELSKLSSKSA KERRNRRKKR    499

rPN4a           KQKELSEGEE KGDPEKVFKS ESEDGMRRKA FRLPDNRIGR KFSIMNQSLL    548
rPN4            KQKELSEGEE KGDPEKVFKS ESEDGMRRKA FRLPDNRIGR KFSIMNQSLL    548
RATNaCh6A       KQKELSEGEE KGDPEKVFKS ESEYGMRRKA FRLPDNRIGR KFSIMNQSLL    549

rPN4a           SIPGSPFLSR HNSKSSIFSF RGPGRFRDPG SENEFADDEH STVEESEGRR    598
rPN4            SIPGSPFLSR HNSKSSIFSF RGPGRFRDPG SENEFADDEH STVEESEGRR    598
RATNaCh6A       SIPGSPFLSR HNSKSSIFSF GDPS-VRDPG SENEFADDEH STVEESEGRR    598
```

Fig. 4B: PN4a/PN4/NaCh6 alignment

```
rPN4a       DSLFIPIRAR ERRSSYSGYS GYSQCSRSSR IFPSLRRSVK RNSTVDCNGV   648
rPN4        DSLFIPIRAR ERRSSYSGYS GYSQCSRSSR IFPSLRRSVK RNSTVDCNGV   648
RATNaCh6A   DSLFIPIRAR ERRSSYSGYS GYSQCSRSSR ISPACAQR-E ANSTVDCNGV   647

rPN4a       VSLIGPGSHI GRLLPEVKID KAATDSATTE VEIKKKGPGS LLVSMDQLAS   698
rPN4        VSLIGPGSHI GRLLPE---- ------ATTE VEIKKKGPGS LLVSMDQLAS   688
RATNaCh6A   VSLIGPGSHI GRLLLR---- ------QRLR WKLRRKALDS -FSFYGPTRL   686

rPN4a       YGRKDRINSI MSVVTNTLVE ELEESQRKCP PCWYKFANTF LIWECHPYWI   748
rPN4        YGRKDRINSI MSVVTNTLVE ELEESQRKCP PCWYKFANTF LIWECHPYWI   738
RATNaCh6A   LRTEGQNQQH NERGHKHASE ELEESQRKCP PCWYKFANTF LIWECHPYWI   736

                      [-----------IIS1-----------]        [-------
rPN4a       KLKEIVNLIV MDPFVDLAIT ICIVLNTLFM AMEHHPMTPQ FEHVLAVGNL   798
rPN4        KLKEIVNLIV MDPFVDLAIT ICIVLNTLFM AMEHHPMTPQ FEHVLAVGNL   788
RATNaCh6A   KLKEIVNLIV MDPFVDLAIT ICIVLNTLFM AMEHHPMTPQ FEHVLAVGNL   786

            --IIS2----------]        [---------IIS3--------]
rPN4a       VFTGIFTAEM FLKLIAMDPY YYFQEGWNIF DGFIVSLSLM ELSLADVEGL   848
rPN4        VFTGIFTAEM FLKLIAMDPY YYFQEGWNIF DGFIVSLSLM ELSLADVEGL   838
RATNaCh6A   VFTGIFTAEM FLKLIAMDPY YYFQEGWNIF DGFIVSLSLM ELSLADVEGL   836

              [--------IIS4--------]             [----------IIS5
rPN4a       SVLRSFRLLR VFKLAKSWPT LNMLIKIIGN SVGALGNLTL VLAIIVFIFA   898
rPN4        SVLRSFRLLR VFKLAKSWPT LNMLIKIIGN SVGALGNLTL VLAIIVFIFA   888
RATNaCh6A   SVLRSFRLLR VFKLAKSWPT LNMLIKIIGN SVGALGNLTL VLAIIVFIFA   886

            -------]
rPN4a       VVGMQLFGKS YKECVCKINQ ECKLPRWHMN DFFHSFLIVF RVLCGEWIET   948
rPN4        VVGMQLFGKS YKECVCKINQ ECKLPRWHMN DFFHSFLIVF RVLCGEWIET   938
RATNaCh6A   VVGMQLFGKS YKECVCKINQ ECKLPRWHMN DFFHSFLIVF RVLCGEWIET   936

                       [-----------IIS6-----------]
rPN4a       MWDCMEVAGQ AMCLIVFMMV MVIGNLVVLN LFLALLLSSF SADNLAATDD   998
rPN4        MWDCMEVAGQ AMCLIVFMMV MVIGNLVVLN LFLALLLSSF SADNLAATDD   988
RATNaCh6A   MWDCMEVAGQ AMCLIVFMMV MVIGNLVVLN LFLALLLSSF SADNLAATDD   986

rPN4a       DGEMNNLQIS VIRIKKGVAW TKVKVHAFMQ AHFKQREADE VKPLDELYEK  1048
rPN4        DGEMNNLQIS VIRIKKGVAW TKVKVHAFMQ AHFKQREADE VKPLDELYEK  1038
RATNaCh6A   DGEMNNLQIS VIRIKKGVAW TKVKVHAFMQ AHFKQREADE VKPLDELYEK  1036

rPN4a       KANCIANHTG VDIHRNGDFQ KNGNGTTSGI GSSVEKYIID EDHMSFINNP  1098
rPN4        KANCIANHTG VDIHRNGDFQ KNGNGTTSGI GSSVEKYIID EDHMSFINNP  1088
RATNaCh6A   KANCIANHTG VDIHRNGDFQ KNGNGTTSGI GSSVEKYIID EDHMSFINNP  1086

rPN4a       NLTVRVPIAV GESDFENLNT EDVSSESDPE GSKDKLDDTS SSEGSTIDIK  1148
rPN4        NLTVRVPIAV GESDFENLNT EDVSSESDPE GSKDKLDDTS SSEGSTIDIK  1138
RATNaCh6A   NLTVRVPIAV GESDFENLNT EDVSSESDPE GSKDKLDDTS SSEGSTIDIK  1136

rPN4a       PEVEEVPVEQ PEEYLDPDAC FTEGCVQRFK CCQVNIEEGL GKSWWILRKT  1198
rPN4        PEVEEVPVEQ PEEYLDPDAC FTEGCVQRFK CCQVNIEEGL GKSWWILRKT  1188
RATNaCh6A   PEVEEVPVEQ PEEYLDPDAC FTEGCVQRFK CCQVNIEEGL GKSWWILRKT  1186
```

Fig. 4C: PN4a/PN4/NaCh6 alignment

```
                [----------IIIS1--------]                          [-------IIIS2
rPN4a        CFLIVEHNWF ETFIIFMILL SSGALAFEDI YIEQRKTIRT ILEYADKVFT
rPN4         CFLIVEHNWF ETFIIFMILL SSGALAFEDI YIEQRKTIRT ILEYADKVFT   1248
RATNaCh6A    CFLIVEHNWF ETFIIFMILL SSGALAFEDI YIEQRKTIRT ILEYADKVFT   1238
                                                                      1236

             ----------------]          [---------IIIS3---------]   [
rPN4a        YIFILEMLLK WTAYGFVKFF TNAWCWLDFL IVAVSLVSLI ANALGYSELG
rPN4         YIFILEMLLK WTAYGFVKFF TNAWCWLDFL IVAVSLVSLI ANALGYSELG   1298
RATNaCh6A    YIFILEMLLK WTTYGFVKFF TNAWCWLDFL IVAVSLVSLI ANALGYSELG   1288
                                                                      1286

             --------IIIS4---------]                       [-------IIIS5
rPN4a        AIKSLRTLRA LRPLRALSRF EGMRVVVNAL VGAIPSIMNV LLVCLIFWLI
rPN4         AIKSLRTLRA LRPLRALSRF EGMRVVVNAL VGAIPSIMNV LLVCLIFWLI   1348
RATNaCh6A    AIKSLRTLRA LRPLRALSRF EGMRVVVNAL VGAIPSIMNV LLVCLIFWLI   1338
                                                                      1336

             -----------]
rPN4a        FSIMGVNLFA GKYHYCFNET SEIRFEIDIV NNKTDCEKLM EGNSTEIRWK
rPN4         FSIMGVNLFA GKYHYCFNET SEIRFEIDIV NNKTDCEKLM EGNSTEIRWK   1398
RATNaCh6A    FSIMGVNLFA GKYHYCFNET SEIRFEIDIV NNKTDCEKLM EGNSTEIRWK   1388
                                                                      1386

                                                                [---
rPN4a        NVKINFDNVG AGYLALLQVA TFKGWMDIMY AAVDSRKPDE QPDYEGNIYM   1448
rPN4         NVKINFDNVG AGYLALLQVA TFKGWMDIMY AAVDSRKPDE QPDYEGNIYM   1438
RATNaCh6A    NVKINFDNVG AGYLALLQVA TFKGWMDIMY AAVDSRKPDE QPDYEGNIYM   1436

             -------IIIS6-------------]
rPN4a        YIYFVIFIIF GSFFTLNLFI GVIIDNFNQQ KKKFGGQDIF MTEEQKKYYN   1498
rPN4         YIYFVIFIIF GSFFTLNLFI GVIIDNFNQQ KKKFGGQDIF MTEEQKKYYN   1488
RATNaCh6A    YIYFVIFIIF GSFFTLNLFI GVIIDNFNQQ KKKFGGQDIF MTEEQKKYYN   1486

                                        [---------IVS1----------]
rPN4a        AMKKLGSKKP QKPIPRPLNK IQGIVFDFVT QQAFDIVIMM LICLNMVTMM   1548
rPN4         AMKKLGSKKP QKPIPRPLNK IQGIVFDFVT QQAFDIVIMM LICLNMVTMM   1538
RATNaCh6A    AMKKLGSKKP QKPIPRPLNK IQGIVFDFVT QQAFDIVIMM LICLNMVTMM   1536

                         [---------IVS2----------]       [-------
rPN4a        VETDTQSKQM ENILYWINLV FVIFFTCECV LKMFALRHYY FTIGWNIFDF   1598
rPN4         VETDTQSKQM ENILYWINLV FVIFFTCECV LKMFALRHYY FTIGWNIFDF   1588
RATNaCh6A    VETDTQSKQM ENILYWINLV FVIFFTCECV LKMFALRHYY FTIGWNIFDF   1586

             --IVS3----------]          [---------IVS4---------]
rPN4a        VVVILSIVGM FLADIIEKYF VSPTLFRVIR LARIGRILRL IKGAKGIRTL   1648
rPN4         VVVILSIVGM FLADIIEKYF VSPTLFRVIR LARIGRILRL IKGAKGIRTL   1638
RATNaCh6A    VVVILSIVGM FLADIIEKYF VSPTLFRVIR LARIGRILRL IKGAKGIRTL   1636

                          [----------IVS5---------]
rPN4a        LFALMMSLPA LFNIGLLLFL VMFIFSIFGM SNFAYVKHEA GIDDMFNFET   1698
rPN4         LFALMMSLPA LFNIGLLLFL VMFIFSIFGM SNFAYVKHEA GIDDMFNFET   1688
RATNaCh6A    LFALMMSLPA LFNIGLLLFL VMFIFSIFGM SNFAYVKHEA GIDDMFNFET   1686

rPN4a        FGNSMICLFQ ITTSAGWDGL LLPILNRPPD CSLDKEHPGS GFKGDCGNPS   1748
rPN4         FGNSMICLFQ ITTSAGWDGL LLPILNRPPD CSLDKEHPGS GFKGDCGNPS   1738
RATNaCh6A    FGNSMICLFQ ITTSAGWDGL LLPILNRPPD CSLDKEHPGS GFKGDCGNPS   1736
```

Fig. 4D: PN4a/PN4/NaCh6 alignment

```
             [-----------IVS6-----------]
rPN4a        VGIFFFVSYI IISFLIVVNM YIAIILENFS VATEESADPL SEDDFETFYE    1798
rPN4         VGIFFFVSYI IISFLIVVNM YIAIILENFS VATEESADPL SEDDFETFYE    1788
RATNaCh6A    VGIFFFVSYI IISFLIVVNM CIAIILENFS VATEESADPL SEDDFETFYE    1786

rPN4a        IWEKFDPDAT QFIEYCKLAD FADALEHPLR VPKPNTIELI AMDLPMVSGD    1848
rPN4         IWEKFDPDAT QFIEYCKLAD FADALEHPLR VPKPNTIELI AMDLPMVSGD    1838
RATNaCh6A    IWEKFDPDAT QFIEYCKLAD FADALEHPLR VPKPNTIELI AMDLPMVSGD    1836

rPN4a        RIHCLDILFA FTKRVLGDSG ELDILRQQME ERFVASNPSK VSYEPITTTL    1898
rPN4         RIHCLDILFA FTKRVLGDSG ELDILRQQME ERFVASNPSK VSYEPITTTL    1888
RATNaCh6A    RIHCLDILFA FTKAVLGDSG ELDILRQQME ERFVASNPSK VSYEAYHTTL    1886

rPN4a        RRKQEEVSAV VLQRAYRGHL ARRGFICRKM ASNKLENGGT HRDKKESTPS    1948
rPN4         RRKQEEVSAV VLQRAYRGHL ARRGFICRKM ASNKLENGGT HRDKKESTPS    1938
RATNaCh6A    RRNEEEVSAV VLQRAYRGHL ARRGFICRKM ASNKLENGGT HRDKKESTPS    1936

rPN4a        TASLPSYDSV TKPDKEKQQR AEEGRRERAK RQKEVRESKC      1988
rPN4         TASLPSYDSV TKPDKEKQQR AEEGRRERAK RQKEVRESKC      1978
RATNaCh6A    TASLPSYDSV TKPDKEKQQR AEEGRRERAK RQKEVRESKC      1976
```

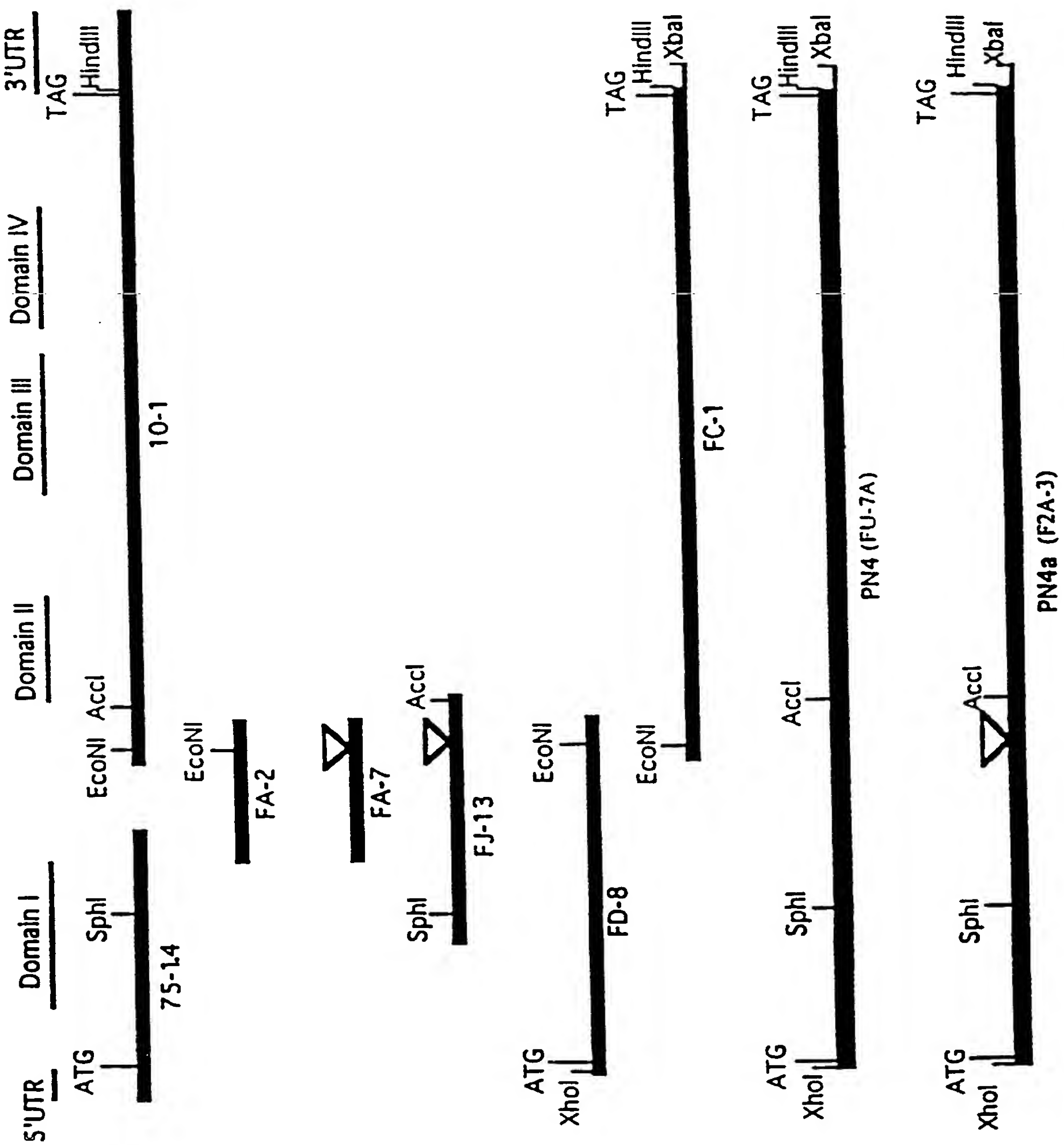
Fig. 5: PN4a/PN4/NaCh6/BrainII Interdomain I/II region comparison

```
rPN4a      DSLFIPIRAR ERRSSYSGYS GYSQCSRSSR IFPSLRRSVK RNSTVDCNGV
rPN4       DSLFIPIRAR ERRSSYSGYS GYSQCSRSSR IFPSLRRSVK RNSTVDCNGV   648
RATNaCh6A  DSLFIPIRAR ERRSSYSGYS GYSQCSRSSR IFPSLRRSVK RNSTVDCNGV   648
rBrainII   DSLFIPIRAR ERRSSYSGYS GYSQCSRSSR ISPACAQR-E ANSTVDCNGV   647
           DSLFVPHRHG ERRP-----S NVSQASRASR GIPTLPMNGK MHSAVDCNGV   653

rPN4a      VSLIGPGSHI ----GRLLPEVKID KAATDSATT-E VEIKKKGPGS LLVSMDQLAS
rPN4       VSLIGPGSHI ----GRLLPE---- ------ATT-E VEIKKKGPGS LLVSMDQLAS   698
RATNaCh6A  VSLIGPGSHI ----GRLLLR---- ------QRL-R WKLRRKALDS -FSFYGPTRL   688
rBrainII   VSLVGGPSAL TSPVGQLLPE---- ------GTTTE TEIRKRRSSS YHVSMDLLED   686
                                                                       698

rPN4a      YGRKDRINSI MSVVTNTLVE ELEESQRKCP PCWYKFANTF LIWECHPYWI   748
rPN4       YGRKDRINSI MSVVTNTLVE ELEESQRKCP PCWYKFANTF LIWECHPYWI   738
RATNaCh6A  LRTEGQNQQH NERGHKHASE ELEESQRKCP PCWYKFANTF LIWECHPYWI   736
rBrainII   PSR-QRAMSI ASILTNTM-E ELEESRQKCP PCWYKFANMC LIWDCCKPWL   746
```

Fig. 6


5'UTR
ATG
Domain I
SphI
75-14
Domain II
EcoNI
AccI
10-1
Domain III
Domain IV
3'UTR
TAG
HindIII
EcoNI
FA-2
FA-7
AccI
SphI
FJ-13
ATG
XhoI
EcoNI
FD-8
EcoNI
FC-1
TAG
HindIII
XbaI
ATG
XhoI
SphI
AccI
PN4 (FU-7A)
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HindIII
XbaI
ATG
XhoI
SphI
AccI
PN4a (F2A-3)
TAG
HindIII
XbaI

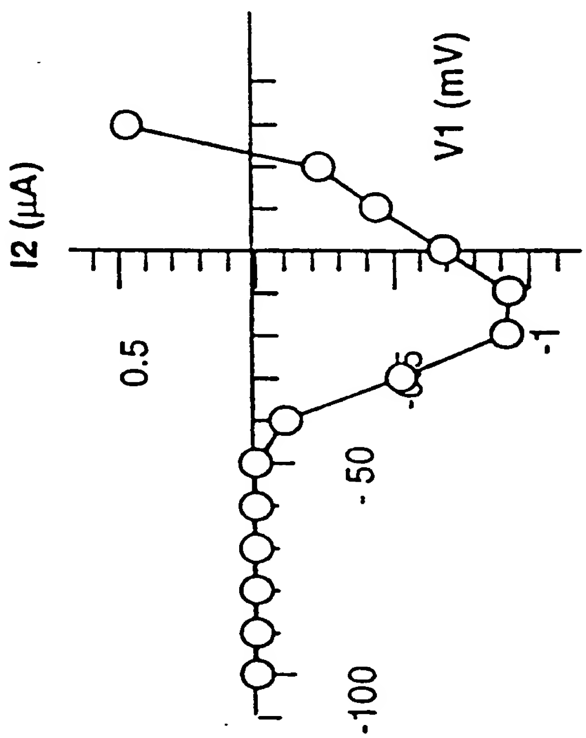


Fig. 7(b)

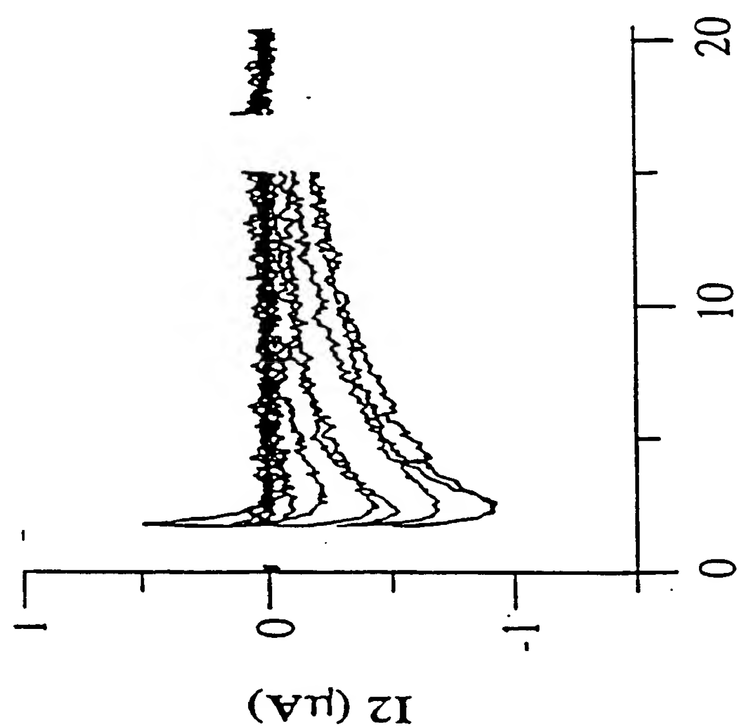
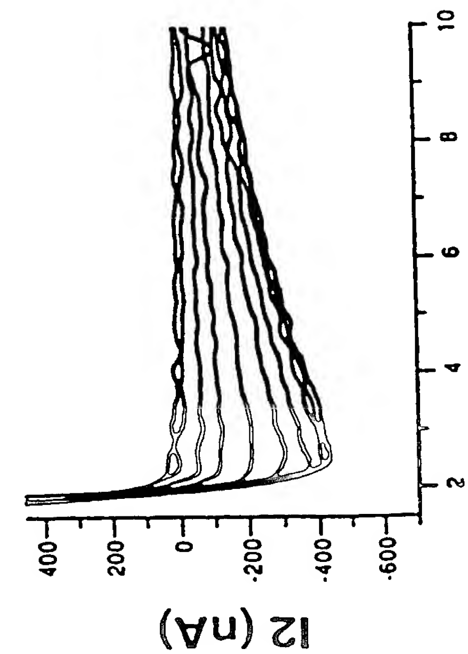
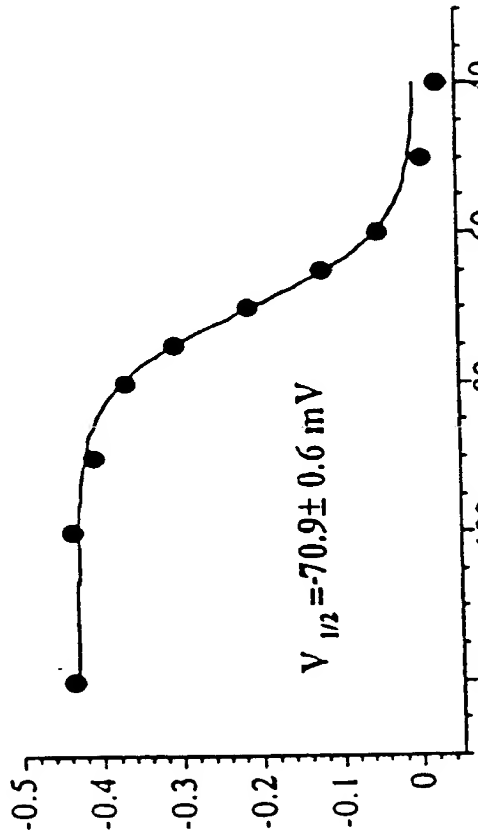


Fig. 7(a)



I2 (nA)
400
200
0
-200
-400
-600
2
4
6
8
10

Fig. 8(a)



$V_{1/2}$=-70.9± 0.6 mV
-0.5
-0.4
-0.3
-0.2
-0.1
0
-120
-100
-80
-60
-40

Fig. 8(b)

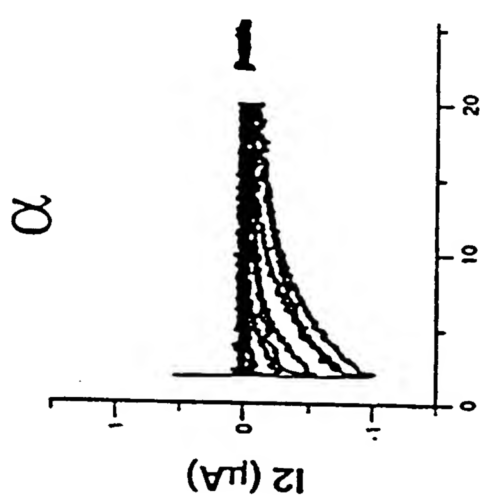


Fig. 9(a)

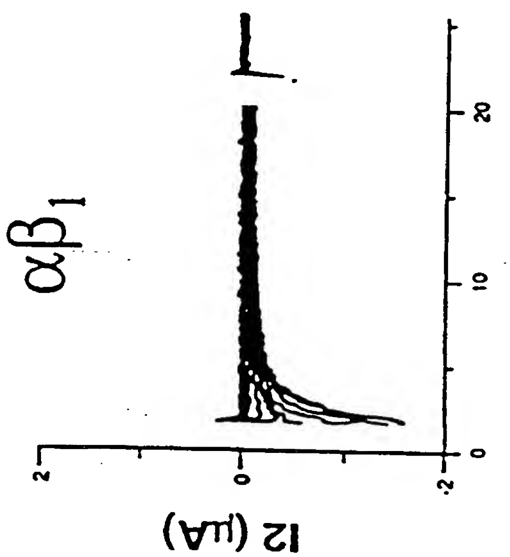


Fig. 9(b)

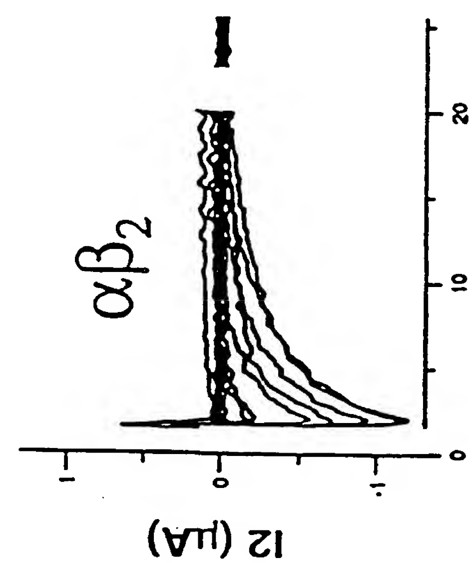


Fig. 9(c)

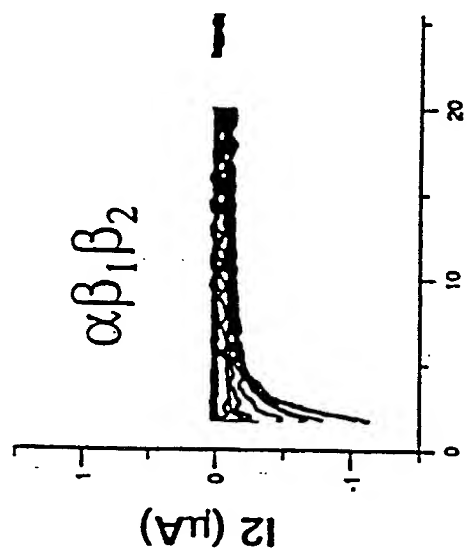


Fig. 9(d)

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(54) Title: TETRODOTOXIN-SENSITIVE SODIUM CHANNEL α-SUBUNIT

(57) Abstract

DNA encoding for a voltage-gated, TTX-sensitive sodium channel is isolated. Also disclosed are polypeptide products of recombinant expression of these DNA sequences, expression vectors comprising the DNA sequence, and host cells transformed with these expression vectors. Other aspects of this invention are peptides whose sequences are based on the amino acid sequences deduced from these DNA sequences, antibodies specific for such proteins and peptides, procedures for detection and quantitation of such proteins and nucleic acids related thereto. Another aspect of this invention is the use of this voltage-gated, tetrodotoxin-sensitive sodium channel as a therapeutic target for compounds.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/00997

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C07K16/28 C12N5/10 C12N1/21
C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KLUGBAUER N ET AL: "Structure and functional expression of a new member of the tetrodotoxin-sensitive voltage-activated sodium channel family from human neuroendocrine cells." EMBO J, MAR 15 1995, 14 (6) P1084-90, XP002069908 ENGLAND see abstract; figure 1 ---	1-50
Y	SCHALLER KL ET AL: "A novel, abundant sodium channel expressed in neurons and glia." J NEUROSCI, MAY 1995, 15 (5 PT 1) P3231-42, XP002069909 UNITED STATES see abstract; figure 2 --- -/--	1-37, 45-50

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 September 1998	11.09.1998
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Gurdjian, D

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/00997

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 380 836 A (ROGART RICHARD B) 10 January 1995 see examples 7,10,11,14 ---	1-37, 45-50
Y	US 5 439 808 A (BLAKE MILAN S ET AL) 8 August 1995 see column 15, paragraph 2 ---	38-44
Y	EP 0 483 113 A (BIO TECHNOLOGY GENERAL CORP) 29 April 1992 see examples 5,13,24 -----	38-44

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International application No.
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INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 50 partly 1-37, 45-49

   tetrodotoxin-sensitive sodium channel DNA sequences proteins with seq.id.1-7 ,vectors, hosts, assays and antibodies .

2. Claims: 50 partly,38-44

   method of growing plasmids containing contructs of tetrodotoxin-sensitive sodium channel proteins employing competent E.coli .

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ational Application No
PCT/EP 98/00997

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